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AN INTRODUCTION
TO THE STUDY OF
INFECTION AND IMMUNITY

INCLUDING CHAPTERS ON

SERUM THERAPY, VACCINE THERAPY, CHEMOTHERAPY
AND SERUM DIAGNOSIS

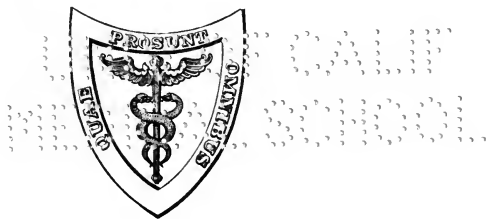
FOR STUDENTS AND PRACTITIONERS

BY
CHARLES E. SIMON, B.A., M.D.

PROFESSOR OF CLINICAL PATHOLOGY AND EXPERIMENTAL MEDICINE AT THE COLLEGE OF PHYSICIANS AND
SURGEONS; PATHOLOGIST OF THE UNION PROTESTANT INFIRMARY AND THE HOSPITAL
FOR THE WOMEN OF MARYLAND; CLINICAL PATHOLOGIST TO THE
MERCY HOSPITAL OF BALTIMORE, MARYLAND

SECOND EDITION, REVISED AND ENLARGED

ILLUSTRATED



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TO
PAUL EHRLICH
THE GRANDMASTER OF EXPERIMENTAL MEDICINE
THIS VOLUME
IS RESPECTFULLY DEDICATED
BY
THE AUTHOR

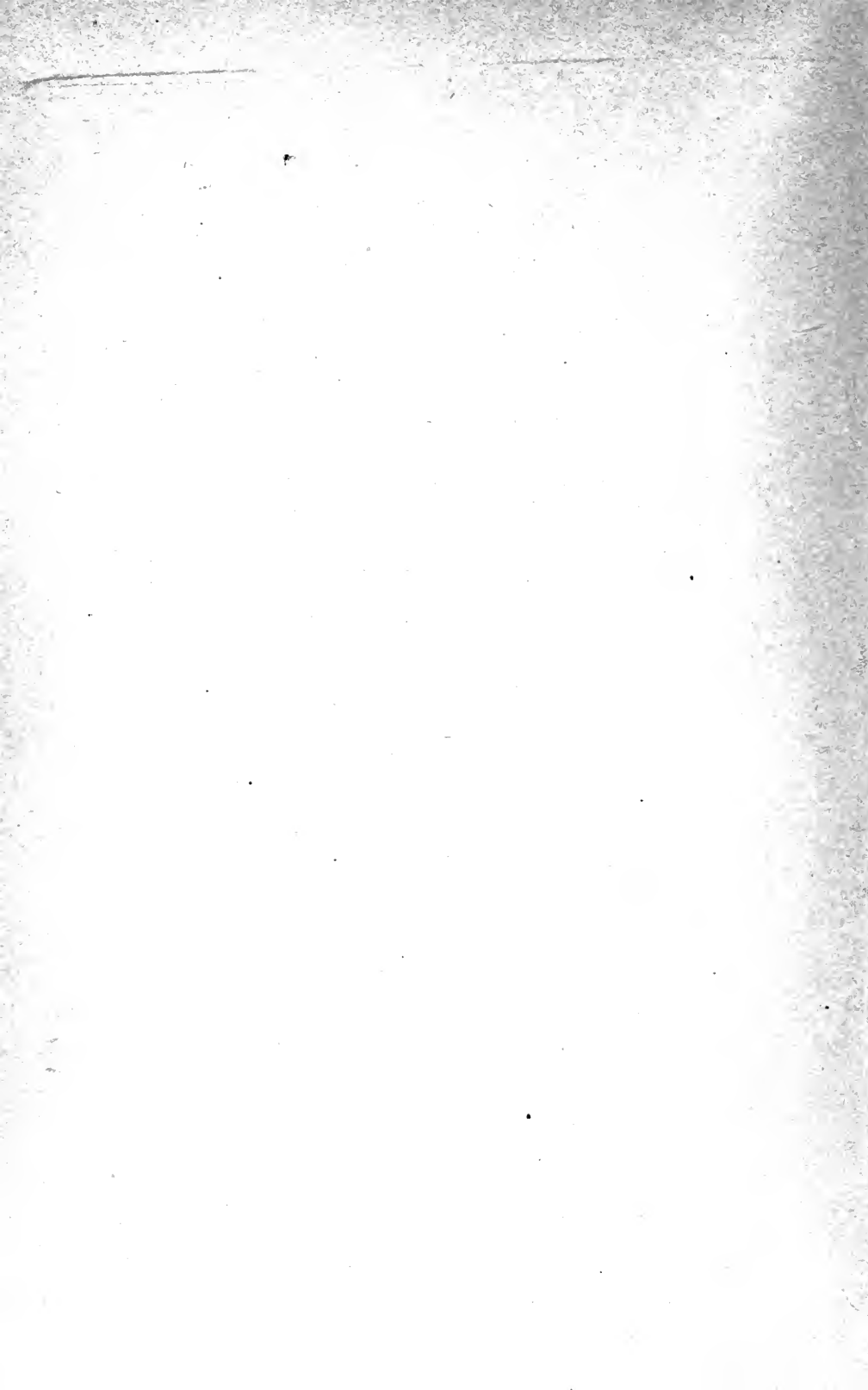
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PREFACE TO SECOND EDITION

THE fact that the first edition of *Infection and Immunity* has been exhausted within a year indicates the interest with which the general practitioner is meeting the marvellous advances of modern experimental medicine. In preparing this new edition the author has endeavored to embody the most notable achievements of the past year, and has thus added sections on auto- and normal serum therapy, on the chemotherapy of pneumococcus infections and of cancer, on the serum diagnosis of pregnancy, etc., besides giving the entire work a careful review.

The author hopes that his work has served to some extent in diffusing a knowledge of this vastly important subject, and in making it readily assimilable and applicable, and he hopes that this new and revised edition may meet with the same generous recognition as its predecessor.

C. E. S.



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INFECTION AND IMMUNITY

CHAPTER I

INTRODUCTION

A SURVEY of the earliest writings in medicine shows that many if not all the diseases with which we have to deal today were existing then. Smallpox, plague, cholera, typhoid fever, dysentery, diphtheria, tuberculosis, malaria, erysipelas, measles, scarlatina, rabies were known to Hippocrates and Galen as they are to us; wound infections existed then as now; nephritis, diabetes, rheumatism, gout, various types of anemia, cancer, etc., occupied the physicians of the days of the Pharaohs as they do those of the present century. This acknowledgment carries with it the admission that during all these centuries the physician has not been able to master these diseases.

Much progress has of course been made, but much more still remains to be done. This is no reflection upon the medical men of the past; they have accomplished their share in the evolution of medical science, and it is unnecessary to point out at this place how well this has been done. Since medical science depends for its own progress upon progress in the subservient sciences, the rapid evolution of modern medicine is the direct result of the wonderful advances in the domain of chemistry, physics, and the various branches of biology. In the dark days of the medieval ages active progress was out of the question, and it is no wonder that therapeutic empiricism sank to its lowest level. Material advance of medical science as a science could only be possible after a foundation had been created, of which anatomy, physiology, pathology, bacteriology, and modern pharmacology are integral components, and as the latter four are the product of the last century almost exclusively, nay even of the last fifty years, there is small cause for wonder that

so little has been accomplished during the many centuries that have passed, and at the same time that so much has been achieved in the brief period that has really been available for productive work.

The days of therapeutic empiricism are fortunately coming to an end. From the standpoint of curative therapy they have brought us but little that is worth retaining—cinchona bark, the gift of the Peruvian Indian, for the treatment of malaria, and mercury, a remedy of the Talmists, as a problematical cure for syphilis. As regards the *curative* treatment of the remainder, not one of the hundreds and thousands of pharmaceutical preparations that have been introduced since the days of the Vedas has been shown to be of value, if as evidence of a curative effect we demand a shortening of that period of time which the animal body itself requires to accomplish a cure. We have learned to *prevent* many diseases by the elimination of the corresponding infecting agents from our midst; cholera, plague, typhus fever, typhoid fever, yellow fever, smallpox, malaria, and diphtheria are diseases which if they still exist among civilized people do so with the consent of the people in the face of a full knowledge of the manner of their prevention.

Wonderful progress also has been made in surgery. By its means countless lives have been saved which otherwise would have been doomed. But after all surgical treatment cannot be regarded as curative treatment in the proper sense of the word; the surgeon may amputate a badly crushed limb or he may remove a diseased appendix, or a cancerous breast, but he does not cure the limb, nor the appendix, nor does he restore the breast to its original condition. The final repair, the healing of the wound, is accomplished by the animal body itself. The surgeon, however, is frequently placed in a condition where he can assist nature materially to accomplish a cure, and in this respect he is certainly more favorably placed than the internist.

The latter may be a most skilful diagnostician, an excellent pathologist perhaps, but he does not cure the diseases with which he is brought into contact. He may in a measure influence some diseases by his directions for the general care of the patient, but, as a rule, the patient dies or recovers irrespective of his therapeutic efforts, insofar at least as these efforts are based upon ancient empiricism. Typhoid fever patients still pursue the same course which was so well

described by the physicians of the medieval ages; our pneumonia death-rate is still what it was when the earliest records on the subject were kept, and is virtually the same for the millionaire in his marble palace, surrounded by doctors and nurses, as for the tramp who is cared for by the roadside by his brother tramps. The "virulence" of an epidemic of scarlatina or measles may vary, but our death-rate in the long run is virtually the same. Where actual progress has been made in the treatment of disease, such progress has been due not to our therapeutic interference by means of drugs, but to a recognition, be it ever so slight, of those factors by which nature herself, unaided and at the same time unhampered by empirical drug treatment, seeks to accomplish that end. For after all the very thing which physicians have sought to accomplish in all the centuries that have passed, viz., the cure of disease, that very thing nature has accomplished by herself, before our very eyes, countless millions of times.

Nature herself cures 75 per cent. of the pneumonia cases, while the physician fails to cure any, for surely he cannot claim as his own what nature does, and he evidently loses the 25 per cent. that nature loses. The fact that nature does not cure all cases could of course be interpreted as indicating that the means at nature's command are after all not perfect. That is naturally a debatable point. So much, however, seems certain that nature's ways, so far as we have become familiar with them, are the only specific ways along which progress seems possible, and that drug treatment, if it ever shall become of value, must start from a different basis, and that basis must be a knowledge of the principles which underlie the interaction between the disease-producing agent and the affected organism.

Immunology.—The study of these forces constitutes the domain of immunology, of which in turn modern chemotherapy, serum therapy, and vaccine therapy are the logical products. The earliest work in this direction is intimately linked with the name of Pasteur, and constitutes the basis of all future work. It was Pasteur who first demonstrated that material progress in the treatment and prevention of the so-called infectious diseases could only be achieved by the recognition of the fact that the production of active resistance to an infecting agent on the part of a susceptible animal necessitates the introduction of the infecting agent into the animal body; in other words, that acquired immunity, be this absolute or relative, temporary or permanent, is merely a phase of infection. The study

of infection then may be regarded as the key-note to the entire problem of the infectious diseases. How does infection primarily take place? how does infection give rise to disease? and how does the animal body overcome infection? these are the most important questions which at present occupy the attention of immunologists the world over, and it is the object of the present work to present to the practising physician the more important data which have already been worked out.

Infection and Infectious Disease.—In the earliest days of bacteriology, when the pathogenic role of various bacteria was just beginning to be understood, it was thought that the presence of such organisms in the animal body could only be anticipated if symptoms of the corresponding disease existed at the same time, or were about to appear; in other words, the presence of a pathogenic bacterium in the body of an individual was looked upon as equivalent to infection, and the terms infection and infectious disease were practically used synonymously. This conception of the terms seemed quite warrantable at the time in view of the findings in such a disease as tuberculosis, where it had just been established that the disease in question was invariably associated with the presence of the tubercle bacillus, while the existence of the tubercle bacillus in the body in the absence of a corresponding lesion was unknown. The majority of physicians hence readily accepted this line of thought, which future investigations have shown to be erroneous. For it was soon demonstrated that pathogenic organisms may be present on the tegumentary and mucous surfaces of the body without concomitant disease.

It is thus well known that staphylococci are present at almost any point of the skin, and that streptococci even may here be demonstrated in perfectly healthy individuals. Pneumococci may be found in the mouths of almost every individual, non-virulent to be sure, in the majority of people, but virulent in fully 15 to 20 per cent. of the cases, in the absence of any symptoms of disease. Streptococci are here likewise not infrequent. Tubercle bacilli have been found in the nasal secretion of healthy attendants on tubercular patients. Diphtheria bacilli are frequently encountered in those who have been about diphtheria patients, and normal carriers of the meningococcus, in districts in which the corresponding disease is prevalent, are frequently more common than patients with the

disease. Then, in the normal intestinal contents there are myriads of bacteria, the majority of them harmless saprophytes, it is true, but in addition there are also staphylococci, streptococci, colon bacilli, and in herbivorous animals the tetanus bacillus and the anthrax bacillus.

Evidently, then, the mere presence of the disease-producing organisms on the tegumentary and mucous surfaces of the body does not indicate either that the individual has passed through the disease in question, or is ill at the time, or is about to fall ill; nor does the mere presence of such organisms constitute infection. If, however, the normal epithelial barrier has once been passed and the deeper structures have been invaded then we can speak of infection, and when infection has once taken place then we *may* also find clinical evidence of such infection, *i. e.*, symptoms of the corresponding infectious disease; but it does not follow because infection has taken place that symptoms of disease *must* of necessity develop.

Infection and infectious disease are thus not synonymous terms. The two may be associated, but they are not necessarily so. Infection probably always results in a disturbance of the normal functions of the host, and if this disturbance rises beyond a certain point, symptoms may develop which constitute what clinicians regard as the corresponding infectious disease. If, however, the normal functional equilibrium of the host is but little affected by the presence of the invading organism, no clinical symptoms of disease develop, notwithstanding the fact that the microorganisms may have multiplied in the body to an enormous extent. There would thus be an infection, but no infectious disease, using the term disease in the ordinary sense of the word. In some cases of this kind, as in anthrax in sheep, for example, the infection may nevertheless result fatally, but the period of time during which the animal shows clinical symptoms of infection is so brief that one can hardly speak of evidence of disease; when this appears death is virtually at hand. In other cases, such as some of the protozoan infections of the blood of various animals (ordinary rat trypanosomiasis, for example), no harm seems to result to the host whatever, even though the blood be swarming with the invaders. The same may at times be noted in partially immunized animals, when extensive infection of the peritoneal cavity may be produced in the case of such organisms, as the

anthrax and the swine plague bacillus, without any concomitant evidence of disturbance of the host. There may thus be infection of a high order without any evidence of associated disease.

Evidently, then, it is necessary to distinguish sharply between (*a*) mere surface invasions, (*b*) infection proper, and (*c*) infectious disease. The first subject belongs essentially to the epidemiologist and the sanitarian, while the study of infection and infectious disease engage the attention of the immunologist.

CHAPTER II

THE NATURE OF INFECTION

IN studying the subject of infection, one of the first questions which naturally suggests itself is: Why does infection not always follow primary invasion? In some cases it might be argued that the invasion at the time of observation was not primary, and that the person in question may have acquired immunity to the micro-organism under consideration at an earlier date, but that the infection at the time was so mild as to have escaped detection. In the case of such organisms as the typhoid bacillus, the plague bacillus, and the cholera bacillus, such an explanation might be warrantable in some cases; but it is evidently an explanation for which proof would be difficult, if not impossible to furnish; it would be a mere assumption without any adequate basis.

Then, again, it might be argued that infection does not occur owing to the existence of a natural *general* immunity; but, as a matter of fact, it is extremely doubtful on the one hand whether an absolute natural immunity really exists among individuals of a species which is known to be generally susceptible to infection with a given organism, and, on the other hand, we find that some individuals actually do become infected at a later date, showing that they were in reality not immune. With such organisms, moreover, as the pneumococcus, influenza bacillus, staphylococcus, streptococcus, and diphtheria bacillus, infection even does not give rise to an immunity that is deserving of the name; on the contrary it leads to hypersensitiveness and not to increased resistance. We can accordingly discard the assumption that a general immunity is an important factor in discussing the reasons why primary invasion does not always lead to actual infection.

Local Conditions and Infection.—On the other hand it is conceivable that *local* conditions may exist which would prevent the penetration of a microorganism into the deeper tissues from certain surfaces, while from others this would be possible. As a matter of fact there

is a good deal of evidence to show that local conditions are of the greatest importance in determining the occurrence or non-occurrence of infection. It is thus well known that infection with the cholera vibrio, the typhoid or the dysentery bacillus can only occur from the digestive tract, while the gonococcus shows a marked predilection for the genital tract and the conjunctiva, and the meningococcus for the upper respiratory tract. The staphylococcus and streptococcus, on the other hand, as well as the plague bacillus may infect from almost any point, and the same probably is true of the pneumococcus, although its special affinity is directed to the respiratory tract. It might be argued, of course, that the organisms in question do not meet with more favorable conditions for infection at the points where this usually occurs than would be the case elsewhere, and that they infect from these points largely because they are the only regions which are usually open to invasion. There is no good evidence, however, to support such a claim, while a number of data go to show that there are unquestionably definite districts which are more prone to become points of infection with specific organisms than others, because of purely local conditions. The gonococcus and diphtheria bacillus are thus incapable of producing an infection through the skin, even when this has been previously wounded at the point of contact with the organisms in question. The cholera vibrio can infect only from the intestinal mucosa, but not from the mouth, the esophagus, the stomach, or the genital tract. In the stomach, indeed, an active multiplication of the organism in question cannot occur, as the cholera vibrio is rapidly destroyed by the hydrochloric acid of the gastric juice.

While local conditions are thus unquestionably of moment in determining a *liability* to infection and primary invasion on the part of an organism, we still have no explanation why pathogenic organisms may exist at these points without consequent infection. The demonstration of certain preferences of localization for the growth of an organism is, however, in itself an important point to establish for unless local conditions were such that the invader could at least maintain itself, subsequent infection would of course be rendered difficult.

Infection from the normal stomach where hydrochloric acid is being produced during many hours of the day, would *a priori* seem to be a difficult matter. In consequence of the active motility of

the organ, however, some of the microorganisms which have been swallowed may readily escape destruction, and on entering the intestinal canal, with its alkaline reaction and numerous nooks and crevices, find suitable conditions for active growth, food material being present in abundance. The main danger to an invader would then evidently come from the numerous saprophytic organisms which have their normal habitat in the very domain in which the newly introduced organism is a stranger. As such it might readily be destroyed or overgrown by the others. If introduced in sufficiently large number, however, the invader could unquestionably maintain itself, for a while at least, and actually become a source of danger, but be destroyed in the end by the normal inhabitants of the bowel. On the other hand, the organism might adjust itself to its new environment, lose its dangerous properties in a measure, and continue to exist without harm to the host. This is probably true of a number of the inhabitants of the bowel which we look upon as normal, such as the colon bacillus, certain streptococci, staphylococci, and others.

Whether or not microorganisms would find the mouth and nasal passages a favorable place for growth under perfectly normal conditions might very well be questioned. The normal secretions which find their way into the mouth and nares are undoubtedly possessed of germicidal properties, which are feeble to be sure, but nevertheless existent, and it is doubtful whether microorganisms could maintain themselves and multiply in those parts which are well irrigated by these secretions. In man, however, there are many nooks and corners where bacteria may lodge and escape the action of the salivary and nasal secretion. The importance in this connection of carious teeth, alveolar disease, the crypts of the tonsils, and pharyngeal and postnasal lymphadenoid structures, etc., can hardly be over estimated. Such districts are notorious breeding-places of microorganisms, and recognized portals of infection. But, after all, while fully realizing that infection is more apt to occur from certain areas than from others, the question still remains unanswered, Why is it that invasion is not invariably followed by infection?

Obstacles to Infection.—The strongest general obstacle to infection no doubt lies in the mechanical integrity of the epithelial lining of the surface of the body and its cavities and ducts, which are in direct or indirect communication with the exterior. This has long

been recognized and is well established. Organisms like the staphylococcus and the streptococcus can thus exist on the intact skin without giving rise to any disturbance whatever; they are evidently unable to penetrate to the deeper structures through their own efforts. The same manifestly holds good for the epithelial structures of the body in general; staphylococci and streptococci exist in the intestinal tract as on the surface of the body without causing any damage. If, however, the epithelial covering at any point is broken and invasion at the point of injury has preceded, or has occurred at the same time, infection of greater or less extent will of necessity follow. In many instances of infection with the organisms in question the break in the continuity of the epithelial covering may be ever so slight, but it is very doubtful whether infection with these organisms ever occurs through an intact epithelial barrier.

Various attempts have been made to prove that infection with *other* organisms can take place in this manner, but the experiments do not carry complete conviction. It has thus been argued that the intact skin must be permeable to an organism like the plague bacillus, because the disease invariably develops in guinea-pigs in which the organism has been rubbed into the shaved abdominal surface. Such experiments demonstrate, of course, that infection with the organism in question may be effected through the skin; but they do not prove by any means that infection takes place through the *intact* skin. Through the mere process of rubbing slight injuries are unquestionably produced, if not directly, then at least indirectly, for we can readily see that the occlusion of hair follicles and the ducts of sweat glands by little plugs of bacteria constitutes an injury just as well as a visible abrasion of the surface. Such little plugs of bacteria may, on the one hand, act as foreign bodies and mechanically damage the more delicate structures with which they come in contact, or the bacteria as such, through their own secretory or degenerative products may cause a local destruction of these more delicate structure and thus open a route to infection of the underlying tissues. This possibility must also be considered in cases where infection takes place apparently directly from the very surface of epithelial linings, but is naturally less likely to play a role in so dense a structure as the surface epithelium of the skin, as in the case of the mucous membranes.

The infection of the urethral mucosa by the gonococcus is fre-

quently cited as an example of the possibility of infection through intact epithelium. But we know that filtrates of gonococcus cultures are in themselves capable of producing a mucopurulent inflammation of the human urethral mucous membrane, so that here again no proof has been afforded that the organism can infect through *intact* epithelium. It is true that the bacterium itself *seems* to be capable of causing the break in the integrity of the epithelium through its own products, but there is also ground for the belief that this break must first occur before actual infection can take place. With such an organism as the gonococcus the question may of course be rightfully asked whether it can ever occur on the mucosa of the urethra without causing infection; in other words, whether primary invasion may ever occur without being followed by infection. The possibility unquestionably exists, and we may then conceive that the organism, in order to multiply sufficiently to cause damage by its own products, must first find a suitable soil for its growth, and that this soil may after all be furnished at some break in the continuity of the epithelial covering.

In the case of the diphtheria bacillus this is very likely, for we know that the toxin production of this organism is fairly constant and that there is no ground for believing that toxin is not formed by those bacilli which may at times be found in the throats of perfectly healthy individuals. As the amount of toxin is, however, evidently not sufficient to cause local necrosis, we must assume that conditions for active multiplication of the organism are not favorable, and we can readily believe that a break in the continuity of the epithelium at some point might be the essential factor which would lead to an actual infection. This break may occur through mechanical means, but it may also occur through the intervention of associated pathogenic agents, and I would emphasize particularly the importance of underlying pyogenic infections, for the occurrence of which the ground is especially favorable in the lymphadenoid structures of the fauces and the nasopharynx.

The importance of such associated infections cannot be overestimated. We have good evidence to show that in their absence, infection with certain bacteria cannot occur at all. It is thus well known that the tetanus bacillus cannot maintain itself, when inoculated by itself into perfectly normal tissues, while the simultaneous introduction of pyogenic organisms renders its growth and multi-

plication possible. In the case of the diphtheria bacillus similar considerations may apply. It must be admitted, however, that mechanical injury is of paramount importance, for we see that the tetanus bacillus, while unable to grow and multiply in structures that are intact, can do so when these have been previously or simultaneously bruised or lacerated. For the reason that the two organisms in question can only exist to advantage in damaged structures, Bail not inappropriately speaks of them as *necroparasites* (necros—dead).

CHAPTER III

THE OFFENSIVE FORCES OF THE INVADING MICROÖRGANISM

WHILE the protection which the macroörganism is afforded by its epithelial covering is thus undoubtedly of great importance, it is a striking fact that infections of *serious* extent are after all comparatively rare, if we consider the frequency with which injury of the tegumentary and mucous surfaces occur. Minor infections, on the other hand, are common enough, and the question naturally arises, Why does not every infection become generalized and lead to the destruction of the host? Evidently this must depend upon one of two factors (sc., an interaction between the two), viz., the nature of the microörganism and the resistance which the macroörganism offers to the presence of the other. Collectively those forces which are at the disposal of the invading organism, and in virtue of which it strives to maintain itself in its new environment, may be termed its *aggressive forces*, in contradistinction to the defensive forces of the host. The former will occupy our attention in the present chapter.

Necroparasites.—Bacteriological examination of the blood during the life of the patient, and of the various tissues after death, reveals a remarkable difference in infections with different organisms. We thus find that certain bacteria, such as the diphtheria bacillus, the tetanus bacillus, and the bacillus botulinus, are possessed of a very low grade of infectiousness, if by this term we mean their power to multiply in the invaded organism. The infection is almost always strictly local during the life of the patient; a general infection is indeed exceedingly rare, and when it occurs it does so only *sub finem vitæ* or after the death of the patient. The tetanus bacillus particularly is practically unable to maintain itself in normal living tissues, and in cases of infection owes its limited development either to the damage done by an associated infecting agent or by direct mechanical injury. Even so, the organism has frequently disappeared

from the body entirely, at the time when the patient is actually dying from the effects of its brief sojourn. Evidently its aggressive forces are minimal, and even though it kills through its highly poisonous toxin, the resistance which the animal body offers to its presence is entirely sufficient to prevent its active development.

In the case of the diphtheria bacillus similar considerations apply, although the organism, after once it has gained a foothold, is not dependent to the same extent upon outside factors for its existence in the tissues. It may be questionable whether it can gain access to the deeper tissues through intact superficial structures, but through its own toxin it is evidently capable of causing marked destruction after once the superficial epithelial barrier has been passed. Associated pyogenic organisms undoubtedly facilitate its growth, but in the deeper structures, at least, their coöperation is not imperative. The diphtheritic exudate may of course extend considerably beyond the original focus of infection, but the infection after all remains a local one in the vast majority of cases. If it becomes generalized at all, this occurs well along toward the fatal end or after death, and is even then usually insignificant. In the case of this organism also the aggressivity is thus not, as a rule, capable of overcoming the defensive forces of the body, while at the same time it is highly dangerous through its toxin. Evidently the infectious and toxic properties of an organism are two independent factors which in the case of the tetanus and diphtheria bacilli bear an inverse relation to each other.

True Parasites.—An altogether different behavior is seen in a group of organisms which is represented by the anthrax bacillus and the chicken cholera bacillus. Here the local infection is followed almost immediately by a generalized infection, the organisms not only maintaining themselves, but actually multiplying freely in the body of the host. Their aggressivity, as compared with the so-called necroparasites, is thus extraordinarily developed, while their toxicity is virtually *nil*. Manifestations of disease are notoriously lacking, while death nevertheless follows. It is remarkable to see rabbits or sheep infected with anthrax bacilli, whose blood is literally swarming with these organisms, quietly feeding and then dying a sudden death without any previous manifestations of disease. We may similarly see guinea-pigs which have been partially immunized against chicken cholera, with the peritoneal cavity a veritable culture

of the organism, without any evidence of disease. Such examples form the exact counterpart to what we see in the case of the necroparasites, but here as there toxicity and infectiousness or aggressivity bear an inverse relation to each other. We see, moreover, that clinical manifestations of disease may be most pronounced on the one hand, even though the infecting organisms have been unable to maintain themselves in the body of the host (tetanus), while, on the other, there may be the most extensive infection without any evidence of a corresponding infectious disease. As Bail has suggested, organisms belonging to this latter class may well be looked upon as *true parasites*, whose aggressive mechanism must evidently be of a different nature than that of the necroparasites previously considered.

Semiparasites.—Between these two extremes stand the semiparasites, which are represented by the cholera vibrio and the typhoid bacillus. Their infectiousness, and hence aggressivity, is already quite well developed, although it is not comparable to what we see in anthrax or chicken cholera, necessitating (in the animal experiment) the introduction of a fairly large number of organisms and often special methods of infection. In *man* the typhoid bacillus is distinctly more aggressive than the cholera vibrio, which latter is rarely found in the blood or tissues, although one would imagine, in view of the extensive epithelial desquamation and superficial necroses, that opportunity for a general invasion would be readily afforded. In addition to their aggressiveness the organisms of this class are possessed of a well-marked toxicity, the effect of which appears quite early in the course of the infection, but does not lead to the production of *specific* symptoms, as we see them in the case of the necroparasites.

Transition Forms.—From the semiparasites the transition to the necroparasites is represented by certain anaërobic butyric acid producing bacilli, such as the bacillus of malignant edema and the bacillus of symptomatic anthrax, which are actively necrotizing toxin producers, but possess a certain degree of aggressivity also, as is evidenced by their wider distribution in the body of the infected animal. Next in order follows the dysentery bacillus which behaves as a typical semiparasite for the guinea-pig, while in the rabbit it is relatively little infectious but exhibits a marked toxin production. The streptococcus and pneumococcus, on the other hand, are closely

related to the true parasites, being characterized by a considerable degree of infectiousness and a low grade of toxicity.

The remaining pathogenic organisms can be readily placed in this system, the determining factors being their aggressivity (infectiousness) and their toxicity. The plague bacillus would thus find its proper position close to the true parasites, while the staphylococcus, meningococcus, and gonococcus would come somewhere between the staphylococcus-pneumococcus group and the semiparasites proper, and so on. It should be borne in mind, however, that the exact position of an organism in this system may vary with different species of animals, at least so far as its aggressivity is concerned. I have pointed out already that the position given the cholera bacillus, for example, is not exactly correct in the case of man, where it should stand close to the necroparasites. The anthrax bacillus in the frog and pigeon has ordinarily no aggressivity whatever, even though the same strain may be most active in other mammals. The factors which produce this difference in behavior are frequently unknown, but sometimes, as in the last example, they are very simple; for in this instance the apparently absolute resistance of the frog and pigeon is referable to the fact that the anthrax bacillus ordinarily does not grow at the temperature which is normal for the animals in question. If, however, one gradually accustoms the organism to those temperatures infection can be produced.

Tissue Parasites.—It will be noted that no mention has been made of the position of either the tubercle bacillus or of actinomyces in the above schema. As a matter of fact, these organisms occupy a position of their own, being essentially *tissue parasites*, while the others may be looked upon as humoral parasites. Their behavior in the macroörganism is in every respect different from that of the remainder. While the latter only affect a certain group of cells (*i. e.*, the leukocytes) in a direct manner, the tissue parasites bring about an altered response of the body at large, *i. e.*, an *allergia* which is in a certain sense characteristic of this group. This peculiar behavior is also shown by one of the animal parasites, *viz.*, the *treponema pallidum*, while the trypanosomes resemble the humoral bacterial parasites. (see also section on *Allergia*).

Virulence, Infectiousness, and Aggressivity.—From the foregoing survey it is clear that the aggressivity of the pathogenic organisms

differs very considerably, and the question naturally arises, To what is this difference due?

Clinically, we have long been in the habit of ascribing the varying severity observed in different cases of the various infectious diseases to differences in the *virulence* of the organism; in other words, the severity of the clinical picture was regarded as an index of the severity of the infection. This conception of the term is no longer tenable, in view of our present knowledge of the relation or rather lack of relation which exists between infection and infectious diseases; for, as we have seen, the anthrax bacillus produces no evidence of disease whatever until the end is almost at hand, although the blood may be swarming with organisms long before.

Using the term *virulence* in the old sense of the word, we would accordingly be forced to look upon every anthrax infection as a non-virulent infection, which would evidently be absurd. On the other hand, we know that in tetanus serious symptoms of disease appear relatively early, even though the bacillus multiplies to a slight extent only, and the infection remains altogether local; in some cases, indeed, the organisms have already disappeared from the body at a time when the patient is dying from the effect of their toxins. Such an infection one would be apt to look upon as especially virulent. Evidently the severity of the clinical pictures is no index of the virulence of the organism. The confusion is altogether due to the fact that in the past the toxic power of an organism and its infectious power have been looked upon as synonymous, while we now recognize that the two are separate factors.

The toxicity of an organism is in a measure an *accidental* property which is of interest from the fact that it is responsible for certain symptoms of the infection, but it is not by any means essential to infection. This is shown especially well in the case of the tetanus bacillus, whose toxins by themselves, after separation from the organism, are capable of producing the identical clinical picture which follows actual infection.

The term *virulence*, in its modern meaning, has reference essentially to the ability of an organism to multiply in the body of the infected animal, and is hence virtually *synonymous with infectiousness or aggressivity*. It is, hence, erroneous to speak of the virulence of a tetanus bacillus or a diphtheria bacillus to indicate the severity of a given case; the clinical picture is essentially due to the action

of toxins, and one should accordingly speak of the toxicity of the organism. Its virulence, *i. e.*, its power to multiply in the body of the infected animal, is always slight. Death is the outcome of its toxic action, but not the expression of an especially high degree of virulence.

The use of the latter term in infections with the necroparasites would only be justifiable if one wished to give expression to the idea that the severity of the clinical picture was due to the formation of an especially large quantity of toxin, which in turn would indicate the presence of an especially large number of organisms. This, however, scarcely enters into consideration, as we know that the necroparasitic toxins are so extremely active that large numbers of organisms are not at all needed to produce disastrous consequences, after primary infection has once taken place.

In infections with the true parasites, on the other hand, the term virulence in its new sense is directly applicable; the more virulent the organism the more readily will it multiply in the body of the infected animal. In the semiparasites the term virulence may occasionally still be applied in its original meaning, and the more justifiably so the more evenly the toxic and the infectious properties are represented in the same organism. By a particularly virulent infection we would here mean an infection, during which there is, on the one hand, an active multiplication, and on the other a correspondingly active toxin formation with the production of a correspondingly severe clinical picture.

Differences in Aggressivity.—Having thus established the proper meaning of the term virulence we may now return to the question, To what factor is the difference in the aggressivity and hence the virulence of the different groups or strains of bacteria due? Two possibilities naturally suggest themselves, which may be operative either individually or conjointly. On the one hand we may imagine that an organism when introduced into the body of an animal which seeks to destroy the invader adjusts itself to its new surroundings by certain changes of a morphological or physiological character, in consequence of which it becomes relatively or absolutely unassailable by the offensive forces of the host, unless, indeed, it already possesses such properties during its saprophytic existence outside of the body. On the other hand we can conceive that the infecting organism actively secretes material which tends to counteract or even to destroy the opposing forces of the host.

Aggressins.—Such substances Bail has termed *aggressins*, and he speaks of aggressivity of this order as *aggressivity in the narrower sense*, while he denotes the former as *aggressivity in the wider sense of the term*. In their places one could substitute the terms *active* and *passive aggressivity*, the latter indicating a passive resistance and the former an actual offensive reaction. The general recognition of the existence of a certain aggressivity on the part of the invading organism is most important. If in the past the attention of medical men has been centred on the defensive mechanism of the invaded organism this interest has been essentially a selfish one. Active progress in the future, however, will depend to a considerable degree upon our knowledge of the defensive forces of the invader. Our present knowledge is as yet quite small, but enough has been learned to establish the importance of further research in this direction.

Passive Aggressivity.—CAPSULE FORMATION.—Among the passive factors the most striking is the tendency to capsule formation, which occurs in some of the pathogenic bacteria, while they exist in the animal body, or when they are grown on media containing animal albumins, such as serum, serum agar, hydrocele agar, milk, etc. When the organism is transplanted to ordinary media this peculiarity rapidly disappears, but can be made to reappear by transferring it to albuminous media, or by reinoculation into the animal body, and so on indefinitely. The most notable organisms which possess this property, aside from the capsule bacteria proper, viz., those organisms which even under ordinary circumstances possess a capsule, such as the bacillus pneumoniae of Friedländer, and the bacillus of rhinoscleroma, are a number of streptococci (str. involutus, vulvitidis vaccarum, mastitidis vaccarum, equi, mucosus), the pneumococcus, the micrococcus tetragenus, bacterium anthracis, bacterium pestis, bacterium cholerae gallinarum, certain pathogenic yeasts, etc. Coincidentally there seems to be an absence of that tendency to form chains which is so common in the case of certain organisms, such as the anthrax bacillus and certain streptococci and pneumococci, when these are grown on artificial media.

In other organisms actual capsule formation has not been observed but in its place an analogous process has been noted, resulting in a thickening of the ectoplasm, so that the bacteria look larger and coarser. This is true especially of the colon and typhoid bacillus and of the staphylococcus, and leads to appearances which often

contrast strongly with the tiny attenuated forms which one is accustomed to see in old cultures on the ordinary media.

The importance of these morphological changes as a defensive mechanism of the bacteria against the opposing forces of the host can hardly be overestimated. It has been conclusively demonstrated, as a matter of fact, that such "animalized" bacteria, as Bail terms them, offer a far greater resistance to the destructive action of bactericidal sera and to phagocytosis than do the corresponding forms which have been cultivated on the ordinary media. That such changes must of necessity lead to a marked increase in the virulence of an organism is of course self-evident. This is well illustrated by an experiment of Horiuchi, who relates that he had in his possession a highly virulent, densely capsulated strain of the micrococcus tetragenus, which resisted phagocytosis almost entirely and killed guinea-pigs in a dose of 100 organisms. When this was grown for a number of days on rather dry agar it lost its capsule-forming power permanently, became readily subject to phagocytosis, and did not affect guinea-pigs even in doses of 1,000,000,000 organisms.

In view of our present knowledge of the relation between capsule formation and virulence, we can now readily understand why *animal passage* of an organism leads to increased virulence. This fact had long been recognized by bacteriologists, but an adequate explanation for it had long been wanting. By starting with a laboratory culture that has been grown for many generations on artificial, non-albuminous media, it may be absolutely impossible to produce an infection at all, even though enormous numbers of bacteria be injected. If injection, however, results we may imagine—in the case of one of the organisms in which capsule formation occurs—that even though the majority of organisms had lost the power of forming capsules, and of thus resisting the offensive forces of the host, a certain number still possessed this property, and that these escaped destruction and multiplied to a greater or less extent.

If then at the height of the infection the animal is killed, or if it succumbs to the infection directly, the now capsulated bacteria will be found capable of successfully infecting the next animal, to which they should be transferred without being first replanted on ordinary media. As a result of the increased degree of resistance which the organisms have acquired in the first animal, they are now in a much

better position to maintain themselves and to multiply in the second, and as the transfers are continued through a series of animals, it will be observed that the number of organisms which is necessary to kill the animal becomes progressively smaller, and the period of incubation, *i. e.*, the interval elapsing between infection and the first evidence of the resulting disease, shorter, until finally a strain is obtained in which the degree of virulence can no longer be increased by animal passage—this constitutes the *virus fixe* in the sense of Pasteur.

Potential Virulence.—While we have thus gained a material basis for our concept of the *actual virulence* of an organism, it is important also to recognize a certain *potential virulence*, *viz.*, the ability of an organism actually to form capsules when placed under conditions which, *cæteris paribus*, are favorable to their development. Evidently only those organisms of the capsule-forming group, or at any rate those in which an hypertrophy of the ectoplasm can occur, are capable of acquiring a notable degree of virulence in which this potentiality is inherent. If once this is permanently lost the organism in question is manifestly non-virulent, so far as its actual development in the infected animal is concerned. We have had an excellent illustration of this in Horiuchi's experiment, referred to above. To determine this potentiality it is sometimes only necessary to grow the organism in serum-containing media and to examine microscopically for capsules. In the non-capsule formers, on the other hand, microscopic examination is insufficient to determine whether an organism under consideration is virulent or not; in that case the animal experiment alone will decide the question.

Factors Determining Capsule Formation and its Significance.—Of the factors which are operative in determining the formation of capsules very little is as yet known. One could imagine, of course, that as the result of favorable changes in nutrition, certain biological changes would result of which the hypertrophy of the ectoplasm is one of the consequences; in other words, that capsule formation is an index of a condition of particularly active nutrition. There are certain facts, however, which suggest that this explanation is not correct. We find that capsule formation may be evoked by agents which have no nutrient properties whatever. Danysz thus found that the anthrax bacillus when grown in arsenical media of increasing concentration forms enormous mucinous capsules which protect

the organism against the bactericidal action of the chemical in question.

Weil and Suzuki further found that certain sarcinae (*i. e.*, absolute saprophytes) when exposed to the action of leukocytes which readily destroy them, undergo a peculiar mucinous degeneration which in the beginning is directly comparable, if not identical with capsule formation, but proceeds beyond this, and ends with the death of the organism. In this case the capsule formation is evidence of a serious impairment of the vitality of the cell and closely analogous to the granular degeneration of vibrios under the influences of the bactericidal substances of the serum. It does not necessarily follow of course that capsule formation among the pathogenic parasitic organisms should likewise be a degeneration phenomenon, but the above observations suggest this possibility, and it will be well in future investigations to take it into account.

It is also quite in accord with the present tendency to regard capsule formation as a pathological state on the part of the micro-organisms that the anthrax bacillus never forms spores in the animal body, no matter how extreme the infection may be. One would accordingly expect that in a long-continued series of transplantations from animal to animal the vitality of the microörganism would finally be damaged so severely that a further transfer would not lead to infection. This actually seems to be the case, for neither Bail nor Gruber and Wiener were able to maintain an uninterrupted series in the case of the anthrax bacillus on the one hand and the cholera vibrio on the other.

In this connection it is interesting to note that in especially severe infections certain organisms, such as the anthrax and the Friedländer bacillus, may appear capsule-free even in the infected body, which at first sight is difficult to reconcile with the idea that capsule formation is essential to infection. The true significance of the phenomenon, however, is suggested by the observation that in the test-tube experiment a serum may be deprived of its power to elicit capsule formation, if an abundant culture has once been raised from it, which seems to indicate that a certain component which is essential to capsule formation has thus been removed. It is similarly possible in the case of certain strains of streptococci to produce either capsulated or non-capsulated generations by varying the intensity of the infection. If then a non-capsulated lot is transferred from the body

of the infected animal to a tube of serum typically capsulated organisms will develop, while the same strain, if grown outside of the body in non-albuminous media, hardly shows any evidence of capsule formation on being transferred to serum. The conclusion hence suggests itself that capsule formation is merely a coincidental evidence of a special state which the organism assumes in the animal body and that its increased resistance in the body is not due solely to its capsule, but to the development of a general infectious ability, of which capsule formation and resistance to phagocytosis are associated, but not necessarily interdependent consequences. Similarly, attenuated organisms of this order are harmless, not merely because they have lost the power to form capsules, but because they are no longer able to assume that special infectious state which *may* be associated with capsule formation. We could accordingly conceive the existence of a special type of immunity which we may term *antiblastic immunity* (Ascoli), which would depend upon such an inability of an organism to develop an infectious state, even in the absence of any *active* antibacterial agencies on the part of the macroörganism.

Organ Virulence.—After the virulence of an organism has been artificially raised, one would imagine that this increase would manifest itself not only in animals of the same species through which it has been passed, but in others as well. This, however, is not the case, and here as elsewhere in immunological work one meets with remarkable examples of specificity for which no explanation can as yet be given. If, for example, the virulence of the chicken cholera bacillus is increased by passage through the chicken, this increase affects this animal but remains unchanged for the guinea-pig. Similarly a certain selective affinity develops for certain organs if the increase in virulence has been brought about through the specific intervention of those organs, and then shows itself irrespective of the manner in which infection is produced. When rats, for example, have been serially infected through the respiratory tract with the lung juice of animals dead with the plague, the virulence of the organisms is not only increased, but plague pneumonia invariably develops on infecting other rats even by the subcutaneous or intraperitoneal route.

Virulence of this order which is specifically directed against certain organs is spoken of as *organ virulence* and is evidently destined

to play an important role in the future study of infection. Capsule formation, however, can scarcely have anything to do with this phenomenon in itself, while we can well imagine that nutritional factors may play an important role. We can thus conceive that during the primary infection, which in turn may be possible owing to capsule formation, a certain group of organisms may have become lodged in a certain organ, and that their vegetative functions here become so modified that the particular juices which are there available can be utilized especially well. If, then, members of this strain are subsequently introduced into another animal, those will develop with special readiness which are placed in contact with the same nutriment to which they had become accustomed in the first host, while the remainder, from lack of this special nutriment, may not develop at all. As a consequence that organ will become the special seat of infection and disease in which conditions for the growth of the organism are most favorable. The affinity for such an organ, may of course be a natural one, and exist already on the part of an organism which has not been passed through an animal for many generations, but there can be no doubt that it may also be acquired.

Attenuation.—The influence of animal passage upon the aggressivity of an organism can thus be twofold—*i. e.*, it may lead to capsule formation, on the one hand, and to a general increase in its functional efficacy as a consequence of especially favorable nutritional conditions, on the other, the outcome being an increased virulence for the infected animal. The reverse will be caused by those agencies which prevent the development of these aggressive forces. We have already pointed out that the ability to form capsules disappears when an organism is grown on ordinary media, and we know that this inability may become permanent; this in itself does not interfere with the viability of the organism as a saprophyte, to be sure, but makes its parasitic existence in the animal body an impossibility. Such a decrease in the virulence of an organism can be brought about in many other ways, although it has not been ascertained to what extent impaired capsule formation is responsible for the change; in some instances this may be the case, while in others this explanation is hardly admissible. Such attenuation in virulence can be brought about by exposure to temperatures which are unfavorable to the growth of the organism; prolonged exposure to the air; exposure to sunlight; increased atmos-

phoric pressure; an electric current; certain chemicals, such as glycerin, carbolic acid, chlorin, trichloride of iodine, potassium bichromate, alcohol, etc., special care being taken, of course, to employ concentrations which will not actually kill the organisms; further, by growing an organism in the presence of others which tend to crowd out the one under consideration; by growth in immune serum, etc.

One additional method deserves consideration, as on first thought its employment might be expected to lead to an increase in virulence instead of the reverse—namely, animal passage. We have pointed out before that the virulence of an organism is thus usually specifically increased for the species employed, while it remains unchanged for other animals; it may happen, however, that this one-sided increase is associated with an actual decrease in virulence for other species. We have a practical application of this principle in the attenuation of the variola virus by passage through the heifer (Jenner), and in Pasteur's immunization against hog cholera by passing the organism through rabbits (weaker vaccine I) and pigeons (stronger vaccine II).

Most important from a practical standpoint is the fact that organisms which have been attenuated in their virulence through one of the methods enumerated, or through still others, that have for their primary object a direct impairment of the organism's resistance, will either not be able to bring about an infection at all, or if this does occur, a modified infection is the outcome with the establishment of a temporary or permanent immunity—a phase of our problem which will be dealt with in greater detail in a later chapter. The essential point to be borne in mind at present is the fact that just as it is possible by artificial means to increase the virulence of an organism, and thus to favor the development of infection, so also is it possible to bring about the reverse, and that the occurrence or non-occurrence of infection must of necessity depend to a very considerable extent upon the presence or absence of certain aggressive forces on the part of the organism, among which the morphological evidence of aggressivity is especially striking.

Active Aggressivity.—I have pointed out previously that in addition to such passively aggressive forces it is quite conceivable that microorganisms may also possess certain active forces, and a great deal of work has actually been done in the attempt to establish their

existence. The true toxins would of course suggest themselves at once as such forces, but as we have seen already, the very organisms in which toxin production is most striking are the least infectious, and they can therefore hardly enter into consideration. We have thus shown that the tetanus bacillus, for example, notwithstanding its active toxin production, is practically unable to maintain its existence in the body following primary infection. If, then, the true toxins are eliminated as *active* aggressive forces, viz., as forces which inhibit the action of the offensive forces of the host, the question arises, What evidence have we that such forces may actually be operative?

With this problem the work of Bail will always remain intimately associated. This investigator found that the peritoneal exudate of animals which had been killed by intraperitoneal injection of multiple fatal doses of such organisms as the typhoid and the cholera bacillus, upon subsequent removal of the organisms and sterilization of the fluid with chemical antiseptics, was capable of transforming subfatal doses of the same organism into fatal doses; in other words, it had acquired properties which evidently favored infection. Bail supposed that definite substances which were secreted by the bacteria in the body of the infected animal, and which he termed *aggressins*, were concerned in the production of this effect. He assumed that the aggressins were substances *sui generis*, largely upon the basis that aggressive exudates in themselves were found to be non-toxic, and when injected into animals by themselves were capable of preventing subsequent infection. This he explained by the assumption that specific reaction products (antibodies)—*antiaggressins*—are formed in consequence of the injection of the aggressins, which render the latter inactive and thus prevent the active invasion of the body by the microörganisms in question (*antiaggressin immunity*).

The aggressive character of the exudates is largely directed against the phagocytes, which, like Metschnikoff, Bail regards as the only true defensive elements of the invaded organism. This he demonstrated by injecting two aggressin-immune animals, *A* and *B*, intraperitoneally with equal doses of a suitable number of organisms, *A* receiving, in addition, a certain amount of aggressin. After the lapse of one or two hours the peritoneal fluid of *B* can then be shown to contain large numbers of leukocytes, and at the expiration of four

hours the exudate is thick, tenacious, milky looking, and is composed almost entirely of polynuclear leukocytes which have taken up many or all of the injected organisms according to the number which were originally introduced. In *A*, on the other hand, the fluid is abundant, relatively clear, poor in cells, but swarming with organisms, few if any of which have been disposed of by phagocytosis. Bail's explanation is that in *B*, where no aggressins have been injected, there was nothing to prevent the immediate inroads of the leukocytes, which was facilitated in fact by the immune condition of the animal, any aggressins that were formed by the bacteria being bound by the antiaggressins already present. In *A*, on the other hand, the antiaggressins were neutralized by the extra injection of aggressins as such, which, moreover, in the presence of bacteria, exercised their negatively chemotactic influence upon the leukocytes, so that bacterial development could go on undisturbed.¹

This interpretation seems quite adequate to explain the function of the aggressins in infections with those organisms, which are notoriously subject to phagocytosis, and in which other destructive agencies on the part of the invaded animals play no role. As will be shown in detail in Chapter VI, however, there are infections, as with the cholera vibrio, for example, in which phagocytosis only plays a subordinate role, but in which the destruction of the organisms is brought about through certain bactericidal substances (bacteriolysins) which are present in the serum. In such cases it is at first sight difficult to see how the aggressins can play a role at all, if, as Bail suggests, their influence is directed almost entirely against the leukocytes. He has pointed out, however, that this is the case, nevertheless, for it can be shown that the leukocytes are capable of rendering harmless the so-called endotoxins which are liberated during the solution of the bacteria (in consequence of the bactericidal sc. bacteriolytic property of the serum), and that by preventing the access of the leukocytes through the agency of the aggressins the animal succumbs to a final intoxication.

As is evident from the above-mentioned facts, the possibility of the formation of special aggressins, in the sense of Bail, is based upon the correctness of the supposition that the substances in question are in reality bodies *sui generis*, and this rests upon the assumption

¹ It is noteworthy that the aggressins by themselves are not negatively chemotactic, but excite hyperleukocytosis.

(a) that they are formed only in the living body of the host, (b) that they are not toxic, and (c) that the immunity which results on injection with aggressin exudates is of a type that is definitely different from the forms which were known before, viz., the antitoxic and the bacteriolytic type.

"Artificial" Aggressins.—A careful investigation of Bail's work has shown that these suppositions were, after all, not well founded. Wassermann and Citron have thus demonstrated that substances with the identical properties of the aggressins of Bail can also be obtained in the test-tube by shaking cultures of various organisms (the swine plague and hog cholera bacillus, for example) with distilled water, proving that the coöperation of the living organism of the host is not essential. The products thus obtained, in contradistinction to Bail's "*natural*" *aggressins*, have been termed "*artificial*" *aggressins*; there is no real difference between the two, however; the quantity is smaller, but with the one as with the other it is possible to transform subfatal doses of bacilli into fatal ones and to bring about a certain type of immunity. The second assumption of Bail that aggressin exudates are non-toxic has also been shown to be incorrect, as the intraperitoneal injection of sufficiently large amounts of dysentery, cholera, and staphylococcus aggressins, in guinea-pigs, will not only cause general marasmus, but actually lead to the death of the animal.

In fine, it has been proved (by the precipitin test, which see) that aggressin exudates contain bacillary proteins, all of which possess a certain degree of toxic action, and cause the formation of certain antagonistic substances when injected into animals. In the light of such knowledge it is now possible to account in a more natural way for those observations of Bail which led him to assume the existence of aggressins as substances *sui generis*. The facilitation of infection is thus readily explained by the fact that the injection of the subfatal dose is accompanied by the simultaneous administration of a certain amount of toxic material, and not of a non-toxic substance, as Bail supposed, so that death is due to the two factors directly and not to the one indirectly. This, however, was nothing new in itself, since Bouchard already had shown that the filtrates of various bacterial cultures facilitated bacterial infection (substances *favorisantes*). More recently Doerr also could prove that both killed cultures of various bacteria and bacterial toxins as such (diphtheria

and cholera toxin) are capable of producing a fatal effect when injected together with subfatal doses of bacteria.

It is also quite clear now why the simultaneous injection of certain bacilli together with suitable quantities of aggressin exudate and corresponding bactericidal (bacteriolytic) serum does not lead to the destruction of the bacteria. Bail assumed an antagonistic action upon the bactericidal substances on the part of his hypothetical aggressins, while the same effect or rather lack of effect is now explained as the consequence of a neutralizing or inhibiting effect of normal bacterial disintegration products (receptors) upon the bacteriolysins. As suitable treatment of animals with bacterial extracts and killed cultures of bacteria leads to the production of a certain type of immunity, in which antitoxins and certain bactericidal substances (bacteriolysins) play a prominent role, and as we have seen that bodies of that order (bacillary proteins and toxins) can actually be demonstrated in the aggressin exudates, it follows that there is no ground for the assumption that an antiaggressin immunity as an immunity *sui generis* exists.

A final point which has been raised against Bail's theory is the fact that in the antiaggressin immune animal (in the sense of Bail) there is no evidence either of increased phagocytic activity or of increased resistance to the multiplication of bacteria. Weil, one of Bail's pupils, has thus shown in chicken cholera infection, for example, that the increase of bacilli in the immune animal may be just as intense as in the control animal two hours before death, while the virulence, as tested on non-immune animals, is unimpaired, and there is no evidence of phagocytosis. While Bail's whole theory of antiaggressin immunity has thus fallen to the ground it must be admitted that in the truly infectious (septicemic) diseases, bacteriolytic immunity likewise does not play a role, and the question hence still remains an open one; how to account for the undoubted immunity which can be produced by repeated injection of animals with so-called aggressins. As the protection of animals, which is thus obtained, is not transferable, *i. e.*, as one animal cannot be rendered resistant (immune) by the injection of blood from an aggressin-immune animal, the question naturally suggests itself, whether, after all, we are not dealing with a type of immunity which is different from the other forms that are commonly recognized. This question will be discussed at greater length in a later chapter; suffice it to say

at this place that there is evidence to show that this type of immunity is essentially an antitoxic immunity, but one in which the antitoxic effect is probably the outcome of structural changes in the chemical make-up of the cell and not the result of a liberation of antitoxic groups from the cell and their action upon toxin molecules in the circulation.

Summary.—To sum up: So far as our knowledge of the actual aggressive forces of the invading bacteria are concerned we must admit that, barring the morphological changes with which we have become acquainted, and which we have come to look upon as passively aggressive forces, active forces furnished by the living organisms during the infection, have not been satisfactorily demonstrated. But we have seen that bacterial decomposition products in themselves possess a certain infection-favoring influence and are in this sense aggressive. That a decrease in the *offensive* forces of the host, finally, is in a measure equivalent to an increased aggressivity of the infecting bacteria is almost self-evident. These forces will be studied in subsequent chapters, but before entering upon their consideration it may not be out of place to briefly review our knowledge of those products of bacterial activity or degeneration which play a role in the production of the picture of the so-called infectious diseases and their probable manner of action.

CHAPTER IV

BACTERIAL POISONS

I HAVE pointed out in Chapter I that the terms *infection* and *infectious disease* cannot be used synonymously. The existence of an infectious disease itself implies the existence of an infection, but infection may exist in the absence of any symptoms which denote disease. In the ordinary trypanosomiasis of rats, for example, there is nothing to suggest that the infected animal is in any way deleteriously affected by the presence of the parasite. There is virtually a symbiosis between the two, from which the host does not derive any evident benefit, to be sure, but at the same time it is clear that the trypanosome on its part does no harm.

In other infections, as in anthrax particularly, harm is actually done, but the symptoms of harm appear so late and are of such brief duration that one is scarcely warranted in speaking of the existence of an infectious *disease*; when symptoms arise death is virtually at hand. In such infections as tetanus, diphtheria, and cholera, on the other hand, symptoms of disease become very evident relatively early after infection, and only too often appall us through their very violence.

On first consideration one might imagine that the severe symptoms in the one group, and absence of symptoms in the other, are merely the expression of a particularly active multiplication of the organisms in the one as compared with the other, and of a correspondingly severe intoxication of the macroörganism with toxic metabolic products furnished by the invading parasite.

This explanation, however, falls to the ground if we remember that in the very group in which the most active and generalized development of organisms occurs, symptoms of disease are virtually absent, while in tetanus and diphtheria the infection is essentially a local one, and the severity of the symptoms out of all proportion to the small number of organisms present. There is, however, a further important difference between the two groups of organisms,

which becomes apparent at once if we inject suitable animals with *killed* cultures of the anthrax bacillus on the one hand, and the diphtheria and the tetanus bacillus on the other. It will then be seen that in the anthrax animal, as before, no symptoms develop, while in the others disease and death occur exactly as though they had been infected with the living organisms. As the same effect is obtained, if the injections are made with corresponding cultures that have been passed through porcelain filters, it is evident that the dead bodies of the bacilli, as such, are not concerned in the production of the result. This is manifestly due to the presence of poisons in the tetanus and diphtheria filtrates and their absence in the anthrax cultures. The existence of a clinical picture of tetanus or diphtheria infection, in other words, the development of the corresponding infectious disease, is thus explained, as are also the negative results in anthrax.

The question now arises: Are all the so-called infectious diseases due to toxic substances derived from the offending parasites? This question can, I think, be answered in the affirmative for those diseases of which the infecting agent is known. Regarding the nature of the toxic agents, however, which are responsible for the symptom-complex of the various infectious diseases, and the mechanism of their action, our knowledge is as yet very meager.

Ptomains.—In the earlier days of bacteriology, when Brieger especially had shown in a long series of elaborate investigations that definite nitrogenous compounds of basic nature and alkaloid-like properties—the so-called ptomains—were formed from animal matter in consequence of bacterial decomposition, and that some of these bodies were poisonous, hope ran high that the application of the same methods to cultures of the pathogenic bacteria proper would lead to the discovery of definite compounds, to which the symptoms of the corresponding diseases could be attributed. These hopes were, however, soon shattered. For a short time, it is true, the discovery of ptomains, supposedly specific of various diseases, was announced from different laboratories. Brieger himself isolated a “typhotoxin” and a “tetanin,” and I well remember, when working in Gautier’s laboratory, translating into French the announcement from a British source of specific ptomains for scarlatina, measles, mumps, etc. Later research then showed that while some ptomains are unquestionably poisonous and *may* occasionally play a role as

pathogenic agents, the group as a whole is of little if any interest from the standpoint of the student of infection and infectious disease.

In the light of more recent knowledge it is even doubtful whether the serious symptoms which are observed in cases of so-called *ptomain poisoning* are in reality due to ptomains. Since we know that a specific organism, the bacillus botulinus, may frequently be demonstrated in "tainted" animal food and that this organism produces a true toxin—not a ptomain—which is almost as active as the toxin of the tetanus bacillus, one not unnaturally feels a little dubious about the role which the ptomains proper are supposed to play in such cases.

The most urgent objection, however, which can be raised against the role of the ptomains as active agents in the causation of the symptoms and pathological changes of the infectious diseases is, above all, the fact that they can never reach such a concentration in the living body as would suffice to bring about a clinical effect. In the course of Brieger's typhoid studies this became especially manifest, for the yield of his "typhotoxin" in typhoid cultures, after four weeks' incubation, was infinitesimally small and often wanting altogether. Noteworthy, further, is the fact that the toxic effect of the isolated ptomains was always markedly less than that of the original culture, and that pathological changes peculiar to infections with the corresponding bacteria have never been produced with ptomains.

To sum up we may say that while ptomains *may* possibly cause disease or even death, as in some cases of cheese or meat poisoning, or in cases where active absorption is taking place from an abscess or a gangrenous focus, there is no evidence to show that they play a role in the pathology of the infectious diseases *per se*, and it is doubtful, to say the least, whether the effect which is commonly attributed to them in the conditions just mentioned is really the outcome of their action.

Toxins.—If, then, the ptomains are eliminated as pathogenic agents the question arises, Are there any other substances derived either directly or indirectly from microorganisms to the action of which the clinical picture of the infectious diseases could be attributed? Three groups of substances are now recognized which are of moment in this connection, namely, the *true toxins* or *exotoxins*, the *endotoxins* and the *bacterial proteins*.

Of these the endotoxins, like the proteins, are part and parcel of

the body of the organisms and are only liberated when these undergo disintegration, while the true toxins are actively secreted by the living cells. This is one of the essential points of difference also which distinguish the exotoxins, or toxins in short, as they are usually designated from the ptomains. The ptomains are products of bacterial action upon certain foodstuffs, and their formation is, hence, possible only when such foodstuffs are directly available, while toxin production is within certain limitations independent of the food supply, and represents a specific function on the part of the micro-organisms in question. The toxin is in a certain sense a product of the anabolic activity of the organism, while the ptomain is merely a katabolic product. The production of a given ptomain, moreover, is not confined to a given type of organism, while true toxin production is specific. Only one organism is known to form diphtheria toxin, only one is known to produce tetanus toxin, and only one is the source of botulismus toxin. That these toxins are actually responsible for the clinical picture of the corresponding diseases is now a recognized fact, and just as the toxins in question are specific products of the bacteria, so also are the symptoms to which they give rise in a large measure specific of the homologous infections.

The tetanus toxin when injected by itself into suitable animals thus causes tonic spasms of the muscles in the neighborhood of the point of infection, increased reflex irritability, dyspnea, increased heart action, and hemolysis exactly as if the animal had been inoculated with the living bacteria instead. The diphtheria toxin produces edema, infiltration, and necrosis at the point of injection, increase of temperature followed by a drop, and in non-fatal doses paralysis and marked emaciation. Botulismus toxin, no matter how introduced, gives rise to external and internal ophthalmoplegia, dysphagia, aphonia, retention of urine and feces, and to respiratory and circulatory disturbances, with absence of fever and of cerebral symptoms, etc.

We may accordingly assume that the toxins in question have a special selective affinity for certain tissues and produce their symptoms in consequence of such affinity. This is seen especially well in the case of the tetanus toxin, which produces its clinical effect through its action upon the central nervous system to which it becomes anchored, as is shown in the following experiment: If 1 gram of guinea-pig brain is triturated with 10 c.c. of normal salt

solution, 1 c.c. of the resulting emulsion is capable of neutralizing as much as 10 fatal doses of the toxin (*i. e.*, fatal for white mice) and of causing a marked decrease in the toxic action of as much as 60 fatal doses of the toxin. The blood, liver, kidneys, spleen, and muscles, on the other hand, do not possess this neutralizing power. The affinity which exists between the hemolytic toxin (staphylolysin) produced by staphylococcus aureus and red corpuscles is similarly shown when the toxin is allowed to act upon the red cells at 0° C., at which temperature hemolysis does not take place; if, then, the corpuscles are thrown down by centrifugation the supernatant fluid will be found to have lost its hemolytic action, while the red cells have taken up the active principle and hold this so tenaciously that it cannot be abstracted again, even on repeated washing with normal salt solution. Other cells are practically inert in this respect. Another toxin produced by the staphylococcus—the leukocydin—has a similar selective affinity for leukocytes.

The activity of the toxins is most remarkable and far exceeds that of the most toxic ptomaines. One preparation of tetanus toxin could thus be shown to be fatal for mice in a dose of 0.00000025 gram, and another in the still smaller dose of 0.00000005 gram. A culture of the bacillus botulinus similarly produced a fatal effect in doses varying between 0.01 and 0.00005 c.c. These figures assume increased significance if we remember that the toxins have never been prepared in a state of chemical purity and that our purest products are still contaminated with a preponderating amount of inert material.

While the diphtheria bacillus, the tetanus bacillus, and the bacillus botulinus are usually mentioned as being the only bacteria which secrete a soluble toxin, it is now known that a number of other organisms also furnish soluble toxins, and there is hence good ground for the belief that some of the symptoms which are observed in the corresponding infections may be referable to such toxins and are in a measure characteristic. The organisms in question are the dysentery bacillus, the bacillus of symptomatic anthrax, the cholera vibrio and closely related organisms (vibrio El Tor, vibrio Nasik), the typhoid bacillus, the pyocyaneus, and the staphylococcus aureus. Of these the dysentery toxin (in the animal experiment) produces paralyses—(especially of the posterior extremities), hemorrhagic diarrhea and subnormal temperature; the typhoid toxin—diarrhea, hyperemia,

and hemorrhages from the intestinal mucosa; the staphylococcus toxin causes hard infiltration and necrosis at the point of injection, besides renal lesions, hemolysis, and leukocytolysis; the toxin of the cholera vibrio, drop of temperature, pareses, rectal prolapse, and death after five hours or later.

Endotoxins.—While the action of the true toxins is thus individually fairly specific, so that one can speak of a hematoxin, a neurotoxin, a leukocytotoxin, etc., or as would probably be more correct: of a hematoxic or a neurotoxic component of a toxin group, this is in a measure, though possibly to a less extent, also true of the *endotoxins*, which, as already explained, are not secreted by the living organisms, but are only set free after the death and disintegration of the parasites. Whether this latter feature is in reality sufficient to warrant such a complete separation of the endo- from the exotoxins may be questioned, particularly since the principal additional differential factor, viz., the inability of the endotoxins to give rise to antitoxin formation on injection into suitable animals, is in the light of recent work no longer recognized. For practical purposes, however, the separation of the endotoxins as a class is, at the present time at least, convenient.

In the earlier days of bacteriology the existence of the endotoxins as substances *sui generis* had been overlooked, and their effect attributed to the action of the bacterial proteins which themselves are toxic to a greater or less degree. Their independent character is, however, now assured by the fact that their injection into suitable animals gives rise to the production of antitoxins, which are capable of neutralizing the corresponding toxins, and that the toxic effect rapidly diminishes on keeping and is seriously impaired by exposure to higher temperatures (55° to 60°), while the proteins resist a temperature of 120° C. for a whole hour. Their action on the living animal, moreover, is totally different from that of the *bacterial proteins*.

Bacterial Proteins.—The bacterial proteins are essentially pyrogenic in character, which property, according to Buchner, is common to most if not to all the bacteria. It has been demonstrated for the staphylococcus, streptococcus, Friedländer's pneumobacillus, the bacillus coli communis, acidi lactici, proteus, prodigiosus, cyanogenes, subtilis, the sarcina aurantiaca, the vibrio of Finkler-Prior, certain water bacteria, etc. In some of the organisms the

pyogenic action does not manifest itself, because death results too early; but it can be demonstrated, nevertheless, if the same organism be tested in less resistant animals. While the chicken cholera bacillus thus kills chickens without evidence of pyogenic action, the injection of sheep, horses, or guinea-pigs leads to the formation of abscesses at the points of injection without a generalized septicemia. This observation in itself goes to show that the specifically toxic effect of the organisms in question is something separate and apart from the pyogenic effect and evidently due to separate substances.

Aside from their general and non-specific pyogenic properties the bacterial proteins in *themselves* are not markedly dangerous to the injected animal, but they have gained new importance, since it has been demonstrated that the introduction of foreign albumins, of whatever kind, leads not to increased resistance (immunity) against such proteins, but, on the contrary, to hypersensitiveness (anaphylaxis, allergy), such that a subsequent injection, after a certain interval of time, may produce the most serious symptoms and even death. As a sensitiveness of this order can very well be imagined to occur in the course of a bacterial disease, the thought has naturally suggested itself that certain symptoms occurring during the later stages of various infections may be explained upon this basis (see section on Anaphylaxis). But even disregarding their possible significance from this point of view, their pyogenic property in itself is sufficient to render them important. Through their attracting effect upon the leukocytes (positive chemotaxis) they immediately assume a clinical interest, and in certain infections no doubt (staphylococcus, streptococcus, colon bacillus) they are responsible for a large portion of the clinical picture (anemia, hyperleukocytosis, pus formation, fever).

Summary.—To sum up then we have seen that the picture of the infectious disease, insofar as the microorganisms themselves are concerned, may be referable (a) to the action of special exotoxins which are actively secreted by the *living* bacteria; (b) to the action of somewhat less specific endotoxins which enter into play only after the death and destruction of the organisms; and (c) to the relatively non-specific action of the bacterial proteins. The mechanism of the action of these various substances will be considered in some detail in a subsequent chapter. At this place it will suffice to point out

that insofar as a direct chemical effect upon the cells of the body is concerned, this can only take place if a mutual affinity exists between such cells and the toxic substances or their derivatives. But it does not follow that because of such a mutual affinity a toxic effect must of necessity result. This can only occur if the combination with the toxic principle implies a toxic effect. If, then, we observe a toxic effect clinically, upon the central nervous system, for example, this does not necessitate the conclusion that the toxin does not act upon other structures of the body also, but clinically the toxic effect is, of course, the only effect which excites our attention.

Remembering the curious interaction between different organs, however, the thought naturally suggests itself that the toxic bacterial products might exert a toxic effect not only directly but also indirectly. This possibility has apparently not received the attention which it deserves, but must nevertheless be borne in mind. It is perfectly conceivable that a toxin might act upon a certain organ in a way to impair its function, without actually endangering the integrity of the cells as such, but that the impairment of its function may carry in its trail secondary effects which become apparent to the clinician at once, while the primary action escapes attention. Every physician is familiar with the effect of various infections upon the gastro-intestinal functions, on the occurrence of constipation defective secretion of hydrochloric acid, etc., factors which we now know to be controlled to a large extent, if not entirely, by hormone action, and it is clear in view of the interdependence of the gastro-intestinal hormones, that interference at any one point in the chain might readily upset the digestive equilibrium and lead to various further disturbances of the metabolism.

Then, again, as I have already pointed out, there is a certain danger from the action of those very products (antibodies) which the body itself forms, primarily no doubt as a defensive reaction, against the products derived from the bacteria and of which more will be said in later chapters. It is clear at any rate that the picture of the infectious disease is unquestionably the composite of more factors than we are apt to think on first consideration, and some of which no doubt will be found to explain such symptoms as the mysterious death from anthrax, for example, where evidences of actual disease are wanting until the end is near, and where the first symptoms are practically the last ones. To explain this point, undue prominence

has been given in the past to mechanical factors. It was suggested that the occlusion of extensive capillary districts with densely matted masses of anthrax bacilli, or, as in some of the severest types of tropical malaria, with masses of plasmodia, was directly responsible for the fatal issue. This purely mechanical element cannot, of course, be ignored, but in the light of our present knowledge of the physiological pathology of the infectious diseases, we are warranted in the belief that the future will bring a more satisfactory explanation.

Infections with Animal Parasites.—While the foregoing considerations apply more particularly to bacterial infections, similar conditions no doubt exist in infections with animal parasites. Primary infection is here often facilitated by the intervention of special infection carriers. We thus know that malaria is transmitted through the bite of infected mosquitoes (*Anopheles maculipennis*), trypanosomiasis through biting flies (*Glossina fusca* and *tachinoides*), African relapsing fever and Texas cattle fever through certain ticks (*Ornithodoros moubata* and *Boophilus bovis* respectively).

With other organisms, such as the *Treponema pallidum* (Spirochete) we may assume the existence of tiny breaks in the continuity of the epithelial covering, as in the majority of the bacterial infections, while with still others, like the ameba coli, we may imagine that the epithelial lining is first destroyed by the parasite itself. What, then, happens, after actual invasion of the deeper structures has taken place, we can only surmise, but it would appear that the aggressivity of the animal parasites is upon the whole even greater than that of the bacteria. A more or less extensive infection apparently occurs in all cases, in which the microörganism has once gained a foothold, some of the organisms in question multiplying in the blood stream (malaria, trypanosomiasis, relapsing fever), others in the tissues (syphilis, amebiasis), only too often without much show of active resistance on the part of the host. What the aggressive forces are which the animal parasite has at its disposal we do not know. In the case of the malarial parasite these are manifestly directed with a remarkable degree of specificity against the red corpuscles. Having once gained an entrance they are evidently perfectly secure; apparently they are open to attack only while they exist free in the plasma.

Of the formation of *toxic products* on the part of the animal parasites nothing definite is known. The clinical history, however, would suggest this. In malaria the occurrence of the chill and fever and

the lack of relation, which exists between the degree of anemia and the number of the parasites, could hardly be accounted for in any other way, while the mere destruction of the red cells itself could be explained by the manifest proteolytic activity of the parasite and consequent changes in the osmotic tension in the cell. In trypanosomiasis the late symptoms at least (sleeping sickness) would suggest a toxic cause, and in relapsing fever the entire symptom complex is toxic in character. As I have said, however, we have no definite knowledge on the subject, which is, no doubt, owing to the fact that until quite recently we were unable to cultivate the parasites in question as we would bacteria, and could hence not study the products of either their growth or disintegration.

CHAPTER V

THE DEFENSIVE FORCES OF THE MACROÖRGANISM

IN the foregoing chapter we have briefly reviewed the aggressive forces of the bacteria and the manner in which they bring about some of the symptoms of the infectious diseases, while we have said nothing as yet of the mechanism by which the macroörganism defends itself against the infection *per se*, and the action of those poisonous products which are so largely responsible for the clinical picture of the infections. This will be our special problem in the chapters which are now to follow. We may here distinguish between those forces which are at the disposal of the animal body at the moment of infection and those which develop only in the course of the infection, and because of the infection. The former comprise the phagocytic forces of the body cells and the normal bactericidal power of the serum, while the second class includes the various antibodies, so-called, viz., those substances which are liberated from the cells in consequence of the introduction into the circulation of cells or cell products which are foreign to the body. In addition we recognize still other defensive factors, which in a measure are operative in a passive way, but which are nevertheless of great importance from the standpoint of immunity.

PHAGOCYTOSIS

While in the lowest forms of animal life phagocytosis is a *sine qua non* for the very existence of the individual, representing as it does the only mechanism by which the animal is capable of apprehending its food, insofar at least as this is of an organized type, this property is lost to a greater or less extent as a *common cellular* characteristic in the higher forms, but is retained by certain cells in all forms of animal life from the lowest invertebrate to the highest vertebrate. In the latter the phagocytic function is, generally speaking, confined to cells which are derivatives of the original mesoderm, the nerve cell being the only apparent exception, on the basis at least that

leprosy bacilli in variable number have been encountered in these cells, and assuming that their entrance occurred through the activity of the nerve cell itself. All other cells in which phagocytosis has been observed are mesodermal derivatives.

Microphages and Macrophages.—Metschnikoff, to whom we are indebted for so much of our knowledge of phagocytosis, in all its aspects, divides the cells which are endowed with this power into two large groups, viz., the microphages and macrophages. The former group is represented practically exclusively by the neutrophilic polymorphonuclear and polynuclear leukocytes, while the mast cells and eosinophiles either do not engage in phagocytosis at all, or do so only to a slight and unimportant extent. The macrophages, on the other hand, comprise the large mononuclear leukocytes of the blood, the endothelial cells lining the peritoneal cavity, the sessile (fixed) mononuclear cells of the splenic follicles and the sinuses of the lymph glands, the stellate cells of Kupffer in the liver, the large mononuclear, so-called alveolar, epithelial cells of the lungs, which latter two according to Metschnikoff are in reality large mononuclear leukocytes; and finally the bone corpuscles and the myeloplaxes or giant cells of the bone marrow.

Phagocytic Function of Various Types of Cells.—What the significance of the phagocytic function of these various types of cells really is in an organism in which so extensive a differentiation has taken place as in the vertebrate animal is largely a subject of conjecture. We may imagine, however, that under normal conditions phagocytosis plays no essential role, and merely represents a general property of mesoblastic protoplasm which is of interest ontogenetically, but of no practical importance. But we can readily see that this same function, even though it remains dormant, while the body is in perfect health, immediately assumes importance of the first order, if foreign cells are introduced from without. Under such conditions the phagocyte is placed in a similar position as its ancestral prototype, the ameba, and it would accordingly display the same or similar functions, of which the phagocytic action is one. In the case of the microphages (polynuclear neutrophilic leukocytes) at any rate we have no evidence that their phagocytic power enters into action under normal conditions, while with the macrophages the disposal of morphological products of normal cell degeneration may possibly be a normal office of the cells in question.

Both types, however, are capable of taking up foreign cells when these are introduced from without, although it lies in the nature of their differing mobility (the granular leukocytes being essentially mobile and the macrophages sessile) that the former play a more important role in the actual conflict between the invading cells and the defensive forces of the host. That the polynuclear neutrophilic leukocytes more especially will take up bacteria and protozoa¹ has been known for many years, and has been demonstrated not only *in vitro*, but manifestly occurs also in the living body, as is suggested by the findings in gonorrheal pus, in the cerebrospinal exudate of epidemic cerebrospinal meningitis, in the peritoneal fluid of general peritonitis, etc., where many of the offending organisms may be found enclosed in leukocytes.

The enormous extent to which phagocytosis of bacteria may go is well illustrated by the following example which came under my observation a few years ago. In a patient who was dying from epidemic cerebrospinal meningitis we could demonstrate the presence of meningococci directly in the stained preparation and calculated their actual number to be 7,380,000 per cubic centimeter. The vast majority of these were enclosed in polynuclear neutrophiles and in large mononuclear cells which I was inclined to view as endothelial cells.

The value of such forces, if they are actually directed against *living* pathogenic organisms in the infected body, is, of course, self-evident. In the earlier days of our knowledge of phagocytosis, when Metschnikoff for the first time insisted upon the importance of the process from the standpoint of immunity, it was argued that the leukocytes were, after all, mere scavengers and could only take up organisms that had already been killed by the bactericidal substances of the serum (see the following chapter), and that their value as a defensive force was thus merely secondary. Metschnikoff and his pupils, however, have demonstrated in a series of investigations that the leukocytes can actually take up living and virulent bacteria in the living host. They showed this for the first time in anthrax-immune pigeons where they were able to recover anthrax bacilli from the leukocytes of the peritoneal exudate, both by culture and animal inoculation.

¹ While this is true, generally speaking, it should be remembered that the phagocytic action of the microphages is essentially directed against bacteria, and that of the macrophages against animal organisms.

Special stress is laid upon the fact that these results were obtained in *immune* animals, as it could only be shown in this way that the leukocytes are capable of taking up not only *living* but also *virulent* bacteria, *i. e.*, bacteria which in the non-immune organism would have produced a general infection.

That living foreign cells are subject to phagocytosis is also well shown by the following experiments: If a guinea-pig is injected intraperitoneally with goose's blood containing the spirillum of Sacharoff, which produces a septicemic infection in geese, and if a drop of the exudate is then examined under the microscope, phagocytosis of the spirilla—in this case by macrophages—can be observed directly, and it will be seen that many of the organisms are quite motile yet with their free ends, while the remainder of the parasites has already been taken up by the cells.

Destruction of Bacteria by Phagocytosis.—While there can thus be no doubt that both microphages and macrophages can take up *living* foreign cells the next question of importance is, What happens to the organisms after they have been taken up? Two possibilities, of course, suggest themselves. We may imagine, on the one hand, that the phagocyte destroys the bacteria, and *a priori* this would seem the most natural thing to expect. On the other hand the possibility at least must be borne in mind that the bacteria may destroy the phagocytes. If this were to happen we could readily understand that phagocytosis might at times be of some danger to the animal, for we could see that a chance might thus be afforded for a wider distribution of the parasites. The possibility of such an occurrence is suggested by the fact that the phagocytosis of tubercle bacilli by giant cells, for example, usually leads to the destruction of the latter and not necessarily to the death of the bacilli. It must be admitted, however, that the greater weight of the evidence goes to show that sooner or later the ingested organisms are killed. The intracellular granular degeneration of bacteria which one can observe directly under the microscope certainly points in that direction.

Of the manner in which the destruction of the bacteria is brought about we are as yet in comparative ignorance. Recent research seems to show that the leukocytes contain special endolysins which may be operative in this direction. This is really what one would expect, remembering that the proteolytic enzymes of the cell can hardly exercise any germicidal action, and that in many of the lower

forms of animal life there is direct evidence of a destruction of the captured living cell preparatory to its digestion. Much work, however, remains to be done in this direction.

While leukocytes are capable of taking up certain bacteria in the absence of blood serum—*spontaneous phagocytosis*—the majority of those organisms which are pathogenic for man and the higher animals become subject to phagocytic action to a notable degree, only after they have been exposed to the action of fresh serum. This fact was emphasized already by Denys and Leclef, who noted that phagocytosis was greatly facilitated if the process were permitted to take place in the presence of serum from an animal that had been immunized against the corresponding organism. In contradistinction to Metschnikoff, who referred this peculiar effect to the possible presence in the serum of substances which exercised a stimulating action upon the activity of the *leukocytes (stimulins)*, Denys and Leclef suggested that the effect of the serum might be directed against the *bacteria* in the sense that the exo- and endotoxins of the latter were neutralized and the organisms thus deprived of their most active defensive weapon against the phagocytic activity of the leukocytes.

Opsonins.—Wright and Douglas then proved that these substances which they could demonstrate in *normal* serum also, actually prepare the bacteria for phagocytosis. This was shown by suspending organisms for a while in fresh serum, washing them with normal salt solution and then exposing them to the action of leukocytes, when phagocytosis promptly occurred, while similar exposure of the leukocytes to serum and subsequent washing gave rise to negative results. Wright and Douglas hence termed the substances in question *opsonins* (from the Latin verb *opsonare*, to cater to, to prepare pabulum for), and expressed the opinion that the opsonins of normal serum and immune serum are identical.

Bacteriotropins.—This, however, is denied by others, such as Neufeld and Rimpau, who confirmed the findings of Denys and his pupils regarding the presence of prophagocytic substances in immune serum and named these bodies bacteriotropins, the essential basis for their belief in the difference of the two groups of substances, at the time being the relative thermostability of the bodies found in the immune serum, as contrasted with the instability of the opsonins of normal serum when exposed to a temperature of only 56° C. for

thirty minutes. Subsequent investigations by numerous observers have furnished additional support to Neufeld's view, the non-specificity of the normal opsonins (established by myself and Lamar as well as by Neufeld, Levaditi and Inman, Ritchie, Russell, and others) being one of the most weighty arguments in its favor.

This is also shown by the observation that the normal opsonins are complex substances, phagocytic action depending upon the joined action of two bodies, viz., a thermolabile component (opsonic complement) and a second component (opsonic amboceptor) which unites with the first mentioned, on the one hand, and the bacterium, on the other, whereas the bacteriotropins of immune sera can act independently (of complement). This difference is shown still further by the different effect which normal and immune sera exercise upon phagocytosis, when virulent as compared with non-virulent organisms are studied in this direction; for, whereas non-virulent strains of staphylococci, streptococci, pneumococci and anthrax bacilli for example, readily succumb to phagocytosis in the presence of fresh *normal* serum, highly virulent forms do so only in the presence of *immune* serum.

Susceptibility to Opsonification.—In this connection it is interesting to note that even aside from the degree of virulence, marked differences exist in the susceptibility to opsonification on the part of different organisms. Gruber and Futaki have thus established three groups in reference to their behavior toward active and inactive normal serum.

GROUP I. Bacteria which are readily taken up by leukocytes in the presence of fresh serum, but not in the presence of inactivated (heated) serum.

Staphylococcus pyogenes aureus.

Streptococcus pyogenes.

Diplococcus pneumoniae.

Bacterium coli.

Bacillus prodigiosus suum.

Bacillus subtilis.

Bacillus erysipelatosus.

Vibrio proteus.

Bacillus diphtheriae.

GROUP II. Bacteria which are readily taken up by leukocytes in the presence of fresh serum, but to a slight extent also in the presence of inactivated (heated) serum.

Bacillus pyocyaneus.

Bacillus sui pestifer.

Bacterium sui septicum.

GROUP III. Bacteria which are not susceptible to opsonic action, and which are hence taken up by leukocytes equally well in the presence of active as well as of inactive serum.

Virulent chicken cholera bacilli.

Virulent Asiatic cholera bacilli.

Wright and Douglas give the following list of organisms as being subject to opsonification:

Staphylococcus aureus and *albus.*

Bacillus pestis.

Micrococcus melitensis.

Diplococcus pneumoniae.

Bacterium coli.

Bacillus dysenteriae (Shiga).

Bacillus anthracis.

Bacillus typhosus.

Vibrio cholerae.

Bacillus tuberculosis.

The diphtheria bacillus (in contradistinction to Gruber and Futaki) and the bacillus xerosis they found to be uninfluenced by the opsonins of normal serum, phagocytosis actually progressing more readily in inactive than in fresh serum.

Role of Leukocytes in Phagocytosis.—Whether or not the leukocytes play an absolutely indifferent role in phagocytosis, uninfluenced by constituents of the serum, still remains an open question. The fact that the leukocytes of a given animal will take up organisms which have been subjected to the action of the serum not only of animals of different species and genera, but of different classes of animals, would on first thought suggest this. But, on the other hand, it has been found that leukocytes, from different animals, show a different phagocytic activity toward organisms that have been opsonified by one given serum. Rosenow could show that in pneumonia the patient's own leukocytes are more actively phagocytic than normal

ones, and that this difference is independent of the action of the serum. As the leukocytes from other infectious diseases (appendicitis and puerperal sepsis) showed a similar behavior toward pneumococci, Rosenow concluded that this difference is essentially the expression of an increased power of resistance and higher activity on the part of the younger cells which find their way into the circulation in acute septic processes, and which in normal blood are in the minority. Differences of this order must unquestionably exist, but they have after all but little to do with the question whether the leukocytes as phagocytes play only a secondary role in the defence of the infected organism against the invading bacteria. The greater part of the evidence is certainly in favor of this view; the existence of substances which directly influence leukocytic action (stimulins, in the sense of Metschnikoff) has not at any rate been satisfactorily demonstrated.

Effect of Opsonification on Bacteria.—Of the manner in which opsonification prepares bacteria for phagocytosis we know nothing that is definite. If we accept the view of Michalis, that ameboid cells react to stimuli, which affect their surface locally, by a local saponification of their lipoid membrane (ectoplasm), and that this leads to local changes of surface tension, which in turn are followed by mechanical surface distortions which we designate as ameboid movements, then we may imagine that the primary effect of the opsonins and tropins upon bacteria may be such that the bacterial surface is so influenced chemically (sc., chemically-physically) that its contact with the lipoid ectoplasm produces the same effect which normally emanates from the body of the leukocyte itself. But this after all tells us very little that is tangible. So much, however, is certain, that opsonification in itself does not impair the vitality of the bacteria.

While the discovery of the opsonins and tropins has materially aided our conception of the general mode of action of the leukocytes, and has demonstrated the relative dependence of the latter upon the presence of the former insofar as the actual process of phagocytosis is concerned, we are still in comparative ignorance of the mechanism by which leukocytes are attracted toward certain bacteria and other organisms. That this actually occurs is a matter of daily observation, every abscess formation being a demonstration of the event; for pus corpuscles are nothing else than polynuclear neutrophilic leukocytes which have emigrated from the bloodvessels to the seat of

infection. In the laboratory the same can be shown by introducing tiny capillary tubes filled with bacterial cultures (staphylococcus, typhoid bacillus, anthrax bacillus, etc.) into the peritoneal cavity of frogs and leaving them for twenty-four hours. At the expiration of this time the tubes are removed and examined under the microscope, when it will be seen that the ends of the tubes especially are filled with leukocytes, the majority of which contain bacteria. Every worker in the clinical laboratory also is no doubt familiar with the precision with which neutrophilic leukocytes will enter the field of vision and sooner or later proceed to devour an extracellular malarial organism which has been left *in situ*.

Chemotaxis.—This property on the part of the polynuclear neutrophilic leukocytes to migrate to a given point at which bacteria or other organisms have entered the body is generally referred to chemotactic influences which the latter exert upon the leukocytes, the term *chemotaxis* being used to designate a certain sensibility on the part of living protoplasm in general to various chemical bodies; it is a characteristic, no doubt, which the leukocyte has inherited from its protozoan ancestors.

According to the type of chemotaxis, *i. e.*, the existence of attracting or repelling influences which chemical substances exercise upon living cells, we speak of *positive* and *negative chemotaxis*. That the latter also may occur in bacterial infections is an established fact, and it is noteworthy that a negative effect may be caused by a virulent strain of the same organism which in a non-virulent condition would produce positive chemotaxis. The important bearing which the type of chemotaxis must have upon the production of an infection is, of course, self-evident. If in a given case, in which the main defence lies in phagocytosis, phagocytosis cannot occur in consequence of negatively chemotactic influences, it is clear that a generalized infection must be the outcome.

Of the nature of the substances which determine the chemotactic effect we know relatively little. Living bacterial cells are manifestly not necessary to this end, for we obtain the same collections of leukocytes in the peritoneal cavity of frogs (see above) with dead organisms and even with the soluble products of bacterial bodies, as with the living organisms themselves, and it has long been known that the injection of sterilized cultures of various organisms will lead to the formation of sterile abscesses (Friedländer's bacillus,

staphylococci, bacillus subtilis, bacillus coli communis, anthracis, prodigiosus, proteus vulgaris, etc.).

Buchner, to whom we owe so much of our information on this subject, was the first to suggest that the chemotactic influences which are here manifestly at work are referable to *bacterial proteins*, and he emphasized that abscess formation is the outcome not so much of the presence of *living organisms*, but of their *dead bodies* and the contained proteins. According to Buchner, moreover, the proteins of all bacteria are positively chemotactic, no matter whether the organisms in question are otherwise pathogenic or not.

Of the mechanism by which negative chemotaxis is brought to bear upon leukocytes we know even less than of the production of positive chemotaxis. That the virulence of the organism plays an important role in this connection is, as I have already indicated, undoubted. We could imagine that those organisms which have developed a certain degree of passive resistance in consequence of capsule formation are less liable to opsonification, and that in consequence phagocytosis either does not occur at all or does so only to a limited extent. In such a case, however, we could hardly speak of negative chemotaxis.

A beautiful example of its actual occurrence, however, is afforded if the attempt is made to increase the aggressivity of certain organisms (in the sense of Bail) by passage through the peritoneal cavity of a series of animals, and transferring with the bacteria some of the peritoneal exudate. It will then be noted that whereas the exudate in the first animal is rich in leukocytes and poor in bacteria, most of which are found within the cells, and whereas a systemic invasion has not taken place, the latter is demonstrable at the end of the series and simultaneously we find the peritoneal cavity filled with a thin serous fluid which is swarming with bacteria, but is almost free from leukocytes. Evidently there were strongly positive chemotactic influences at work in the beginning of the series, while at the end negative chemotaxis controls the situation.

The problem then seems to resolve itself into a question of difference between the material injected into the first as compared with the last animal of the series. The bacteria *per se* can here be left out of sight, as the same result is obtained if for each injection the original culture is employed. The only point of difference then is the absence of aggressive exudate in the first animal of the series and its presence

in the subsequent animals, and as we have come to look upon Bail's aggressins as being nothing more than endotoxins which have been set free after the death of the organisms, we are forced to the conclusion that the negatively chemotactic effect must be referable to such substances. In this sense a certain parallel undoubtedly exists between the virulence of an organism and the chemotactic effect which it produces.

Phagocytosis as a Defensive Factor.—From the foregoing considerations it is clear that the leukocytes can rank as defensive factors only in the presence of opsonins or tropins, and providing that the aggressivity of the invading organisms is not above a certain level; it accordingly follows that any factor which tends to lower the normal content of opsonins or prevents the prompt formation of tropins will virtually be equivalent to an aggressive influence and simulate an increased virulence on the part of the infecting organisms. The recognition of this possibility offers an explanation of the formerly more or less obscure *modus operandi* of some of those factors which the clinician speaks of as predisposing causes of disease.

Everyone is familiar with the serious course which pneumonia is apt to take in drunkards and with the liability to staphylococcus infections of diabetics. Both are types of infection in which phagocytosis plays a prominent defensive role, and we have already sufficient evidence to show that both alcoholism and diabetes tend to lower the opsonic content of the blood. In such cases we could readily understand that an increased virulence on the part of the infecting organism, would, in itself, not be necessary to produce the infection or to favor its generalization. This, indeed, seems to be Wright's attitude in reference to those infections in general, in which phagocytosis is the mainstay of defence on the part of the body, for he expressed the belief that primary infection occurs in consequence of a lowered opsonic content of the blood, in contradistinction to the idea that the opsonic content drops because of the infection.

On the other hand, we can now understand why hyperemia at the point of infection should be of use in combating the infection, no matter whether the hyperemia be the direct outcome of the bacterial invasion or produced artificially (Bier's method; counter-irritation by cauterizing sinapisms, applications of iodine, turpentine, etc.).

Variation in Opsonic Content of Blood.—That the opsonic content of the blood does not remain constant after infection has once begun

seems to stand to reason. We may imagine that in infections of sufficient magnitude the normal opsonins are immediately used up to a greater or less extent, and that their new formation as well as the production of specific tropins then begins. We know but little, however, of the mechanism by which this is brought about, and by which the quantity to be produced is regulated, not to speak of the origin of the bodies in question. As far as the latter point is concerned, some observations which I made, together with Lamar, led us to look upon the leukocytes themselves as the possible source of the opsonins, but the evidence was not conclusive. On the other hand there can be but little doubt that the production of opsonins and tropins is caused in consequence of the absorption of bacterial products. This phase of the subject has been thoroughly investigated by Wright and his pupils, who found that the injection of killed cultures (vaccines) of various bacteria will increase the opsonic content, if employed in suitable amount, whereas overdoses will cause a diminution.

This observation throws light upon the remarkable fluctuations in the opsonic content of the blood that have been noted by many observers during the course of various bacterial diseases. We may well imagine that these fluctuations are brought about by irregular absorption of bacterial products, and it naturally suggests itself that in those diseases particularly which are characterized by a certain chronicity, and in which the opsonic content tends to be low (tuberculosis, acne, furunculosis, gonorrheal arthritis, etc.), it might be possible to raise the latter by artificial means (vaccination) and thus to influence the infection in a favorable way. This assumption, however, presupposes that an increased content of normal opsonins or the appearance of specific tropins would be the only factor lacking to insure adequate phagocytosis and thus an eradication of the infecting organisms; but, as I have already mentioned, there is evidence to show that with *virulent* bacteria at least, in which the virulence is due to capsule formation, phagocytosis may not take place even though opsonins or tropins be present in abundance, and it is indeed possible that this factor may be responsible for some of the unsatisfactory results which vaccination has thus far yielded in the *curative treatment* of bacterial diseases.

CHAPTER VI

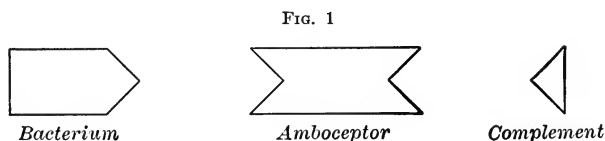
THE BACTERICIDAL SUBSTANCES OF THE BLOOD

WE have seen in the foregoing chapter that the outcome of a bacterial invasion will of necessity be influenced by the phagocytic defence of the body, but that this in turn is largely dependent upon the presence of certain auxiliary factors in the body fluids. The recognition of the interdependence of these two elements is most important, as it has in a measure served to unite the two opposing factions of immunity students, between which a deadlock had practically developed, viz., the cellular school, headed by Metschnikoff, and the humoral school of Pfeiffer and Ehrlich, who looked upon the phagocytic activity of the leukocytes, and certain bactericidal properties of the body fluids respectively, as the essential protective mechanism of the body against bacterial infection.

Alexins.—That the blood serum *per se* really possesses active bactericidal properties had been demonstrated already by Fodor, Nuttal, and Buchner. The latter ascribed the bactericidal action of blood serum to substances which he assumed to be of the nature of ferments, and which he designated as alexins. Subsequent studies, which are intimately associated with the names of Ehrlich and Morgenroth, Bordet, Neisser, and Wechsberg, etc., have then shown that the bactericidal action of the serum is dependent upon the presence of two substances, one of which serves as a connecting link between the bacteria and the second substance, and which has been variously termed *intermediary body* (Ehrlich and Morgenroth), *substance sensibilisatrice* (Bordet), *fixateur* (Metschnikoff), but which is now generally spoken of as *amboceptor* (Ehrlich), whereas the second substance has been designated as *alexin* (Buchner and Bordet), *cytase* (Metschnikoff), or *complement* (Ehrlich and Morgenroth).

Of these two substances the complement is thermolabile and destroyed by heating for 30 minutes at 56° C., while the amboceptor is relatively thermostabile, being rendered inactive only at a temperature of 68° to 70° C. The complement itself is incapable of combining

with the bacteria, whereas the amboceptor is readily anchored to the organisms. This can be shown by treating serum with killed bacteria (of suitable kind) at a temperature of 0° C., and subsequently removing these by centrifugation. The absorption of the amboceptor is then shown by the fact that such serum is no longer capable of causing the destruction of living organisms of the same order, while the addition of such extracted but fresh serum to inactive (heated), non-extracted serum will render this actively bactericidal. The rationale of this will be readily understood by reference to Fig. 1 and bearing in mind the relative thermostability of the amboceptor as compared with the complement.



Mechanism of Interaction between Bacteria, Amboceptor, and Complement.—Much of our knowledge of the mechanism which is involved in the interaction between bacteria, amboceptor, and complement has been obtained from a study of the closely corresponding globulicidal (hemolytic) properties which certain sera possess for red corpuscles of animals of alien species. Working with washed corpuscles, the compound character of the hemolysin, and the absorption of the amboceptor by the cells, can be very well demonstrated as follows: Red corpuscles from an animal of a suitable species are washed free from serum with saline (by centrifugation), and then suspended for a couple of hours in an actively hemolytic serum, the mixture being kept at a temperature of from 0° to 3° C. They are then thrown down again by means of the centrifuge, when the supernatant fluid is tested at body temperature, on the one hand against untreated washed corpuscles, and on the other against those used in the extraction. In the latter case hemolysis will result because the corpuscles have absorbed the hemolytic amboceptor and are now subjected to the action of the complement, union with which evidently does not occur at the low temperature at which the extraction was carried out. In the case of the untreated corpuscles no hemolysis is observed, because the amboceptor has been previously removed by the corpuscles used in the extraction, showing also that

complement alone possesses no hemolytic properties. That amboceptor by itself is similarly inactive is proved by the fact that the treated corpuscles *per se* will not be hemolyzed when these are suspended in saline.

Demonstration of Bactericidal Substances in Serum.—The mere demonstration of the presence of bactericidal substances in a given serum is a relatively simple matter, while the study of the actual extent of its destructive action meets with difficulties, which are largely owing to the fact that blood serum, while it may be bactericidal, is at the same time a very favorable culture medium, and that within certain limits bacterial destruction and bacterial reproduction go hand in hand. The values which are thus obtained are hence of necessity relative values only, and in reality merely express to what extent cell destruction predominates over cell formation.

Whether or not a given serum contains bactericidal substances can be determined either by direct microscopic examination, *i. e.*, by inoculating tiny little tubes of fresh serum with very small amounts of a certain organism (cholera vibrio, best) and observing the resultant suspensions in the hanging drop after a brief incubation at 37° C., or by intraperitoneally inoculating a guinea-pig weighing about 200 grams with a very small quantity of an agar culture (less than $\frac{1}{100}$ milligram = $\frac{1}{20}$ oese—in suitable dilution—in the case of a virulent culture of the cholera vibrio), when specimens of the peritoneal fluid are removed by means of glass capillaries at intervals of 10, 20, or 30 minutes, etc., and examined either directly in the hanging drop or after staining, in the dried smear. With either method it will be observed that the organisms at first lose their motility and then contract to little granules which in the beginning are highly refractive, but gradually become paler and paler, until they dissolve altogether. At first the granules can still be stained with anilin dyes, but as the process of destruction proceeds they become paler and paler, until at last they are no longer demonstrable. As Wassermann very appropriately remarks, the granules melt away like wax in boiling water.

Pfeiffer's Experiment.—Still more striking results will be obtained if the animal is simultaneously injected with a small quantity (about 1 milligram) of serum from another animal which has been previously rendered highly immune against the organism in question by vaccination (which see). In such a case a much larger quantity of bacteria

may be injected (1 oese) with impunity, and it will be observed that notwithstanding the large dose no bacteria will be found in the peritoneal cavity at the expiration of one hour (Pfeiffer's experiment). The same result will be obtained if instead of using a normal guinea-pig and injecting some immune serum together with the bacteria, these are introduced by themselves into a previously immunized animal.

The reason why bacteriolysis will be so much more extensive in the presence of immune serum is the fact that as a result of infection (vaccination, immunization) the amboceptor content of the blood serum is materially increased. The complement which is necessary for the experiment is normally present in the living animal. In its absence, of course, bacteriolysis could not take place, and as complement readily becomes inactive outside of the body, after standing even for a relatively short time at body or room temperature, it is essential if the experiment is conducted *in vitro* that only perfectly fresh serum be used. Otherwise bacteriolysis will not occur, even though the serum may be rich in natural amboceptors.

Quantitative Estimation of Bactericidal Substances.—The quantitative estimation of the content in bactericidal substances of a given serum is most conveniently carried out by starting with a suspension, of known number, of the organism to be examined, and inoculating tubes containing known amounts of serum, after which these are incubated for a certain period of time and plates are prepared in which the number of surviving organisms is finally determined by a direct count. As serum is in itself an admirable culture medium for most organisms it is, of course, essential to reduce this factor as much as possible in the experiment. To this end one can either determine the total number of bacteria which is *completely* killed by a given amount of serum in a given length of time, or one can determine the extreme degree of dilution in which a given serum will still exercise a bactericidal effect, or one may determine the maximal bactericidal effect, which is observed after different intervals of time. The general arrangement of such a test is apparent from the following example, which is taken from Wright, and which represents the titration of a given serum against cholera vibrios on the one hand and typhoid bacilli on the other:

A. Determination of the Strength of the Bacterial Emulsion which Serves as a Basis of the Experiment.—The emulsion in question is

diluted in the proportion of 1 to 100,000 and 5 c.c. of the resultant dilution distributed over the surface of plates. The number of developing colonies is then counted. The result shows the following:

Type.	5 c.c. of a 1 to 100,000 dilution contain:		1 c.c. of the undiluted emulsion hence contains:
Cholera vibrios	Plate I	20.0 organisms	
	Plate II	21.0 organisms	
	Average	21.5 organisms	410,000 organisms
Typhoid bacilli	Plate I	21.0 organisms	
	Plate II	30.0 organisms	
	Average	25.5 organisms	510,000 organisms

B. *Estimation of the Bactericidal Strength of the Serum.*—Equal quantities (1 c.c.) of the undiluted serum and of various dilutions of the original emulsion are mixed and after twenty-four hours' exposure plated out, when the number of developing colonies is counted.

Type.	Dilution of the bacterial emulsion.	Number of developing colonies.	1 c.c. of serum thus kills:
Cholera	undiluted	1	
	1 to 10	0	
	1 to 100	0	About 410,000 organisms
	1 to 1000	0	
	1 to 10000	0	
	1 to 100000	0	
Typhoid	1 to 10	8	
	1 to 100	1	
	1 to 1000	0	About 5100 organisms
	1 to 10000	0	
	1 to 100000	0	

A good idea of the progress of bacteriolysis, and the gradual gain of reproduction over destruction, when the amount of serum was insufficient at the start to kill all the organisms, as also of the differing resistance which different strains of organisms offer to the destructive forces of the serum, may be had from the study of the following table (taken from Trömmersdorff). The figures in general represent the variations noted in different tests; those of the *a* series were obtained with a typhoid strain that had been freshly

cultivated from a patient, while the *b* series has reference to a common laboratory strain.

	Original count.	After 2 to 3 hours.	After 5 to 6 hours.	After 24 to 25 hours.
Series <i>a</i> . . .	10221 to 12256	2074 to 4275	3217 to 4664	8344 to 39125
Series <i>b</i> . . .	2016 to 6586	0 to 0	0 to 0	0 to 0

Specificity of Normal Antibacterial Amboceptors and Complement.

—Practically important is the fact that the normal thermostabile antibacterial amboceptors are specific, as can be shown by treating an inactivated serum with cholera vibrios, for example, when it will be noted, after removal of the organisms by centrifugation, that the fluid, upon the addition of suitable complement, has lost the power of causing the destruction of newly added cholera organisms, while it is still destructive for typhoid organisms. In other words, the anticholera amboceptors have been extracted, while the antityphoid amboceptors have not been affected by the first extraction.

Detailed analytical studies of the different kinds of amboceptors contained in normal human serum are apparently lacking, but it appears from what has been done that whereas anticholera, antityphoid, anticolon, and antidysentery amboceptors are usually to be found, normal blood possesses no bactericidal power over the various staphylococci, the pneumococcus, micrococcus melitensis, bacterium pestis, bacillus xerosis, and the diphtheria bacillus, nor do such substances appear in the human being as the result of infection.

Providing that suitable complement is present, bacteriolysis will, of course, occur whenever blood serum containing a given amboceptor is brought in contact with the corresponding bacteria. As a general rule the complement of the same serum as that containing the amboceptor, or at any rate that from an animal of the same species, will be found to be effective, but there are a number of curious exceptions. The serum of the human being, of the ox, and of the dog thus contains anti-anthrax amboceptors, which are not activated by the corresponding complement. Such sera *per se* have no bactericidal action whatever for the organism in question, while the addition of a little rabbit serum renders them active. The recognition of the fact that the serum of an animal of a different species may contain complement that will activate a given amboceptor is of great practical interest, as it is often technically more convenient to use as complement the blood of a certain animal rather

than that of another; but it is essential to remember that considerable differences in the behavior of such sera exist, and that the sera from certain animals only will serve to activate others.

These interrelations (worked out for hemolytic amboceptors on the one hand and bacteriolytic amboceptors on the other, both normal and immune) are shown in the following table:

Type of cell.	Amboceptor-containing serum of the		Corresponding complement found in the
Red cells of rabbit	Dog	(normal)	{ Guinea-pig Ox Goat Sheep
Red cells of rabbit	Ox	(normal)	{ Guinea-pig Rabbit Rat
Red cells of guinea-pig . . .	Ox	(normal)	{ Guinea-pig Man Rat Horse Sheep (less markedly)
Red cells of sheep	Rabbit	(immune)	Guinea-pig
Red cells of chicken	Rabbit	(immune)	Guinea-pig
Dysentery bacillus	Goat	(normal)	Horse
Typhoid bacillus	Rabbit	(normal)	Guinea-pig
Proteus	Dog	(normal)	Rabbit
Proteus	Cat	(normal)	Rabbit
Anthrax bacillus	Dog	(normal)	{ Rabbit Rat
Anthrax bacillus	Man	(normal)	{ Rabbit Rat Horse
Anthrax bacillus	Ox	(normal)	
Anthrax bacillus	Pig	(normal)	
Anthrax bacillus	Goat	(normal)	
Vibrio Metschnikoffi	Chicken	(immune)	Pigeon
Vibrio Metschnikoffi	Goose	(immune)	{ Pigeon Rabbit Goat
Vibrio Metschnikoffi	Goat	(immune)	Rabbit
Vibrio cholerae	Goat	(immune)	Rabbit
Dysentery bacillus	Horse	(immune)	Man
Typhoid bacillus	Man	(immune)	Rabbit
Typhoid bacillus	Donkey	(immune)	Rabbit
Typhoid bacillus	Horse	(immune)	Rabbit
Typhoid bacillus	Rabbit	(immune)	Goat
Typhoid bacillus	Dog	(immune)	Guinea-pig

Origin of Bacteriolytic Amboceptors.—Regarding the origin of the bacteriolytic amboceptors, our knowledge is as yet very meager. The researches of Pfeiffer and some of his pupils suggest that the spleen is possibly more actively concerned in their production than any other organ, but there is also evidence to show that they may be formed in the tissues at large.

Relative Importance of Amboceptor and Complement.—As to the relative importance of amboceptor and complement, opinions differ. Ehrlich regards the amboceptor merely as an indifferent connecting link between the bacteria and the complement, and Bordet also views the complement as the essential factor in bacteriolysis, the amboceptor playing the role of a mordant or activator; on the other hand, Pfeiffer emphasizes the greater importance of the amboceptor, and likens its role to that of a proferment with the complement playing the role of the corresponding kinase. In support of this view he calls attention to the fact that as a result of immunization (infection, vaccination) only the amboceptor is increased, while the complement content is not affected, and further, also, that during the process of hemolysis (which is in every respect closely related and directly comparable to bacteriolysis) the complement is active, not in proportion to its absolute amount, but in accordance with its concentration; this would be quite in harmony with the supposition that its action in reference to the amboceptor is essentially that of a catalyzing agent.

Origin and Structure of Complement.—Regarding the origin and structure of the complement our knowledge is likewise imperfect, though somewhat more definite than that concerning the amboceptor. While originally it was viewed as a single substance, Ferrata has shown that on dialysis the complement separates into two components, one of which is carried down in the precipitate of globulins—the so-called *middle piece* (Mittelstück), while the other remains in solution—the *end piece* (Endstück). Of the two, as the term indicates, the first named (Mittelstück) unites with the combination of bacteria (sc., blood corpuscles) and the corresponding amboceptor, while the end piece only exercises its activity after this union has been effected. Either fraction alone possesses no bactericidal properties in the presence of a suitable amboceptor, though it appears that either component can in a measure supplement the action of the other, in the sense that a very small quantity of the globulin fraction

of the serum (middle piece) is sufficient to effect bacteriolysis, providing that a sufficiently large amount of the end piece is present; and *vice versa*, the germicidal properties of the end piece are enhanced by increasing the amount of the middle piece. Since the middle piece of different animal sera is interchangeable, the thought suggests itself that the two complement components in the living animal are actually present as separate substances. The apparent absence of complement in various sera which we have noted above, might thus be due to the absence or deficiency in the end piece only, rather than to actual absence of complement as a whole.

Complementoid.—This conception of the duality of the complement is quite in accord with the original view of Ehrlich and Morgenroth regarding its structure, according to which the substance in question contained a combining (haptophoric) group which effects the union with the amboceptor and a second (toxophoric or zymophoric) group to which the action of the complement is essentially due. When this latter group is destroyed, while the first remains active, so-called complementoid results, which would mean in more modern parlance that the middle piece remains, while the end piece has been destroyed or altered so as to be rendered inactive.

Chemical Nature of Amboceptor and Complement.—Regarding the chemical nature of amboceptor and complement our knowledge is very meager. Liebermann has pointed out that a certain analogy exists between the action of amboceptor and oleic acid. Oleic acid and hog serum together will thus cause the almost instantaneous hemolysis of hog corpuscles, while the serum by itself is inactive, and the same quantity of oleic acid alone brings about the lysis of the corpuscles only very slowly. This observation is suggestive, but can hardly be taken to prove the identity of the hemolytic amboceptor and oleic acid, especially in view of the highly specific action of the various amboceptors.

Complement, on the other hand, has been viewed as a compound of albumin with a lipoid (Noguchi and Liebermann). Noguchi thus found that mixtures of soaps and inactivated guinea-pig serum, while inactive by themselves, caused the hemolysis of red corpuscles which had been previously treated with amboceptor. He himself, however, points out that the action of such artificial complement is materially slower than that of the native serum. But notwithstanding this and other points of similarity between active and artificial complement,

such as the spontaneous disappearance of the complementary properties on standing, inactivation at 56° C., absence of a hemolytic effect at 0° C., etc., the proof that complement is in reality a lipoid-albumin product has not yet been furnished.

Origin of Complement.—Regarding the origin of the complement, Buchner and Metschnikoff both thought that it was derived from the leukocytes, but while Buchner looked upon the substance as a secretory product of the living cells, Metschnikoff claimed that complement is only formed when the cell dies, during the process of blood coagulation; and that it does not exist preformed in the circulating blood. An enormous amount of labor has been expended to support this view of Metschnikoff on the one hand and to disprove it on the other. As a result it may now be regarded as a fairly well established fact that the normal body fluids contain free complement even when there is no evidence that leukocytic degeneration has taken place.

The long discussed question, also, whether or not the blood *plasma* contains free complement, may now be answered in the affirmative. On the other hand, there can be no doubt that bactericidal substances can be extracted directly from the leukocytes. This can be shown in the following manner: An aseptic exudate is produced in animals by the intrapleural injection of aleuronat, when the cellular elements, which are mostly polynuclear leukocytes (Metschnikoff's microphages), are thoroughly washed with saline, repeatedly frozen and thawed and the resultant material allowed to stand at body temperature. After a while it can then be shown that this extract is quite rich in bactericidal substances and, like the fresh blood serum, loses its action on exposure to higher temperatures. But on comparing the behavior of such leukocytic extracts with normal bactericidal sera certain points of difference appear, nevertheless, which suggest that the substances which are operative on the two sides may not be identical.

Apart from the different temperature at which inactivation takes place and the slower action of the leukocytic extracts which, moreover, can progress in the absence of neutral salts (contrary to the bacteriolytic sera), it is especially noteworthy that certain organisms, such as the cholera vibrio and the typhoid bacillus, which are very susceptible to the action of bacteriolysins, are hardly affected by leukocytic extracts. The latter, moreover, contain no substances

which are capable of activating inactivated anticholera or anti-typhoid sera, *i. e.*, sera containing the corresponding bacteriolytic amboceptors, but deprived of their active complement.

We are thus forced to conclude that the leukocytic origin of complement has not been proved, but we are also forced to admit that no other cells have as yet been shown to form complement. As long as the latter was regarded as a single substance this negative search might very naturally lead one to think that our technique may have been imperfect, but now, when we know that what we call complement is very evidently composed of two constituents, which can be separated from one another and then reunited to reform active complement, the possibility suggests itself that these two components may have a separate origin, and it will accordingly be necessary to repeat the search from this standpoint.

Leukins.—Since the leukocytes have been virtually eliminated as the source of the serum complement, in the older sense of the word, while their bactericidal action toward certain organisms at least is an established fact, we are forced to the conclusion that the body has at its disposal still other defensive factors than those with which we have thus far become acquainted. To what extent such substances occur in the tissues at large still remains to be determined. *A priori* it would seem reasonable to expect that they might be present in all cells, but thus far their production by the leukocytes only has been satisfactorily established.

These *leukocytic alexins*, as we may term them, using the word alexin in the original sense, *viz.*, synonymously with "protective substances," have been variously described as *leukins* and *leukocytic endolysins* by Schneider and Peterson respectively, and the former seems to have proved quite conclusively that these bactericidal substances are actually secreted by the leukocytes, as Buchner originally claimed for the common alexins of the serum. This was demonstrated by placing leukocytes for 30 minutes in diluted blood serum (5 per cent. in normal salt solution), when it could be shown that the solution had developed very active bactericidal properties. During this process the leukocytes were not destroyed, but could be made to furnish additional, equally active extracts without impairment of their vital functions (power of phagocytosis, locomobility). If the same experiments were carried on in an atmosphere of carbon dioxide the solutions developed no bactericidal properties, while a trans-

ference of the leukocytes to ordinary conditions again led to an active production of "leukins," the cells having apparently not been damaged by their exposure to the carbon dioxide. Corresponding results were obtained with Bier's congestive lymph, and satisfactory proof thus furnished that these bodies are formed *in vivo* as well as *in vitro*. Schneider, hence, naturally concludes that the beneficial effect obtained with this method of treatment is probably due to the increased production of these substances.

What the determining factor is that causes the secretion of leukins is unknown, but we may well imagine that a special *stimulus* may here be operative, and that such substances may be liberated from the tissues at large under various conditions which need not necessarily be pathological.

While the above-mentioned bactericidal substances, namely, the bacteriolysins of the serum and the leukins or endolysins of the leukocytes, unquestionably exist as such in the plasma, another substance or group of substances which may likewise be viewed as protective agents or alexins, in the wider sense of the word, are formed only during the process of coagulation from the blood platelets, as has been satisfactorily demonstrated by Gruber and Futaki. Their action, however, seems to be directed almost exclusively against the anthrax bacillus and its congeners.

Summary.—To sum up then: we have become acquainted with various defensive agents on the part of the animal body, any one or all of which may become operative after infection has once occurred, *i. e.*, after a given organism has penetrated through the external epithelial barriers of the body; and knowing some of the offensive weapons of the invaders, we can form a conception of the manner in which systemic invasion may take place or in which it may be prevented. A great deal will, of course, depend upon the quantitative relations existing between the offensive and defensive factors which are engaged in the strife.

Offensive-defensive Mechanism in Infections with Necroparasites.—If the invader is actually open to attack at the point of infection by those agents which are at the disposal of the host, a successful resistance is at least possible, which *may* carry with it the recovery of the infected individual. This, however, is not necessarily the case. For we have seen already that some organisms, such as the tetanus bacillus, are capable of producing poisons of such potency that

infinitesimally small quantities are sufficient to produce death; whose manner of action, moreover, is such that the focal infection may long have ceased to exist, while the toxin is being conveyed to and brought into contact with cells, damage to which leads to the death of the patient.

In an infection of this sort the local superiority of those defensive forces of the host with which we have thus far become acquainted, over the purely vegetative forces of the invader, is evidently totally insufficient to preserve the life of the infected individual, unless, indeed, the disproportion between the number of the infecting germs, and the protective forces at the point of attack should be so greatly in favor of the latter, that no multiplication of the bacteria occurs at all, and then only provided that the number of invading organisms is so small that the amount of toxin which they could secrete before being killed would be insufficient to cause death. Theoretically this possibility could certainly exist. Whether it enters into consideration practically is beyond our knowledge.

This type of infection illustrates two points very well, viz., that the destruction of the invading bacteria at the point of entry does not necessarily prevent the development of symptoms of systemic disease and even of death, and that the protective forces with which we have thus far become acquainted are inadequate to counteract the deleterious influence of toxins of this order.

Offensive-defensive Mechanism in Infections with True Parasites.—

In infections with organisms like the anthrax bacillus the situation is altogether different. The picture which is here seen has been analyzed with great care by Bail, whose account I am here following in some detail.

If a guinea-pig or, still better, a rabbit is injected intraperitoneally with a moderate amount of a broth culture ($\frac{1}{4}$ to 1 c.c.) of the anthrax bacillus, and small specimens of the peritoneal fluid are removed from time to time, it will be observed, after a short while, that the bacilli show external marks of degeneration, and are being extensively taken up by the leukocytes, which have appeared in large numbers, and undergo intracellular degeneration.

Evidently some of those defensive forces with which we have just become familiar (opsonins, alexins) are here at work, and unless the number of organisms injected has been too large, these normal protective forces are apparently sufficient to successfully combat the

infection, for it will be observed that after a certain length of time the peritoneal cavity is seemingly free from bacteria and may remain so for twenty-four hours or longer. Conditions are, however, in reality not at all so favorable as appearances would lead one to think, for presently organisms begin to reappear and to multiply rapidly in spite of the fact that leukocytes are present in abundance. Phagocytosis then no longer occurs and signs of extracellular degeneration are altogether wanting. Simultaneously bacilli have appeared in the circulating blood and multiply here also without hindrance.

It might be argued that this change in conditions was brought about through a gradual loss of those defensive substances in the peritoneal fluid of the guinea-pig, to which the primary successful resistance was due; but this is disproved by the fact that if a new lot of "culture" bacilli, like those used to bring about the infection in the beginning, be now injected, these will be destroyed as readily and in the same manner as the first. Evidently, then, the same defensive factors of the host are still available.

The conclusion, hence, suggests itself that some change may have taken place in the bacteria, and microscopic examination shows, as a matter of fact, that the newly developed brood really differs from the stock culture in having become encapsulated. Further experiments, however, show that the capsule formation in itself does not explain the result, for if some of these capsulated bacilli are injected into the peritoneal cavity of another guinea-pig, they do not immediately multiply without hindrance; but, as in the beginning of the first experiment, they undergo extensive extracellular degeneration, and here, as there, the peritoneal cavity may be temporarily freed of bacteria, although phagocytic destruction apparently does not take place.

This shows clearly that while the phagocytic forces are no longer available in combating the capsulated organisms, some of the normal extracellularly active lytic forces are still available. But, if so, why do they remain inactive in the body of the first animal? It can easily be shown that guinea-pig serum in itself has little or no bactericidal power, so far as the anthrax bacillus is concerned. If, then, the peritoneal exudate has this to a marked extent the thought naturally suggests itself that the destruction of the bacilli may be the outcome of a combined serum-leukocyte effect—possibly in the sense of Schneider's leukins or Peterson's leukocytic endolysins.

If Schneider's experiments permit the inference that these substances are formed through the secretory activity of the living cells, then the possibility also suggests itself that this activity may be impaired or paralyzed as a consequence of bacterial action, and this is what Bail actually claims for his aggressins. He has shown, moreover, that the peritoneal exudate has lost its anthracocidal properties even before animalized bacteria are there demonstrable, and that organisms are at this time already present in the internal organs and notably the spleen. He accordingly develops the following picture of what actually happens: In consequences of the normal protective forces, *i. e.*, the phagocytic action and the secretion of leukins on the part of the attracted leukocytes, the majority of the injected organisms are killed off soon after their introduction. Some of them escape, however, and in the internal organs and notably the spleen these find a relatively safe refuge. Here they assume their "animalized" (infectious) state and begin the secretion of aggressins. The latter are distributed through the general circulation and also reach the peritoneal cavity, where they inhibit the secretory activity of the leukocytes and then furnish suitable conditions for the multiplication of any surviving bacilli that may yet be present. The animal is then stripped of its entire defensive mechanism and now succumbs to the generalized infection.

That the peritoneal fluid actually contains substances which can prevent the liberation of leukins, at the time of the second invasion, Bail has demonstrated beyond a doubt. For, on adding peritoneal exudate from an infected guinea-pig, obtained at a time when the primary destruction of the bacteria has been followed by their reappearance in encapsulated form, to a mixture of normal serum and leukocytes, in certain definite proportions, it can be shown that the bactericidal effect of the leukins is completely suspended. If the cells contained in this mixture are, however, killed and simultaneously extracted by alternate freezing and heating to 56° C., the resultant solution is again bactericidal, showing that the active substances are not injured by the aggressin exudate, but that their formation is merely impeded. The reason, then, why the encapsulated organisms can at first develop in the body of a *fresh* animal is to be sought in the primary absence of aggressins, which only develop in sufficient quantity after a certain length of time.

When this point has been reached, the animal is void of all defen-

sive measures, as the capsulated organisms which alone are present are not susceptible to phagocytosis, and as bactericidal substances are no longer formed, owing to the paralyzing effect of the aggrassin upon the leukocytes, so that boundless multiplication and a general invasion of the body are the outcome.

If the infection of the guinea-pig is started subcutaneously instead of intraperitoneally the picture is somewhat different. In this case a primary destruction of the organisms, comparable to what occurs in the peritoneal cavity, is not seen; on the contrary, there is active multiplication from the start. The explanation of this difference is no doubt to be sought in the greater difficulties which would present themselves to a prompt collection of cells and serum at the point of attack.

In either event the infection, when once it has started, progresses without resistance and ultimately leads to the death of the animal. How this is brought about is unknown. So much, however, seems to be certain that unlike the infections with the so-called necroparasites (tetanus, diphtheria, botulismus) toxins do not play a role in anthrax, and we can accordingly only say that the fatal end in infections of this order must result in an indirect way. Significant in this connection is the fact that anthrax infection in animals that are naturally somewhat resistant, or in others in which a certain degree of resistance has been artificially produced, is followed by symptoms of actual disease and a gradual decline in health until death ultimately occurs.

Similar considerations apply to infections with streptococci and possibly also with pneumococci. While culture streptococci readily succumb to phagocytosis, the animalized organism is highly resistant. But while the anthrax bacillus (in the absence of aggrassin) is readily destroyed by living leukocytes aphagocitically (*i. e.*, without phagocytosis, through the agency of the liberated leukins), this does not occur in the case of the streptococcus. Against this organism the body apparently possesses no defence excepting the phagocytic function of the leukocytes, and this the truly infectious streptococcus can overcome only too readily through the agency of its aggrassins. The importance of the latter will be appreciated, if we bear in mind that a streptococcus exudate, which has been rendered cell- and bacterium-free by centrifugation, is capable, in suitable quantity, of completely inhibiting the bacteriotropins of a

corresponding immune serum and of thus preventing phagocytosis and bacterial destruction through this agency.

In this respect there is a striking difference between the truly infectious and the non-infectious or merely locally infectious streptococcus, for on adding aggrassin from an infectious to a non-infectious strain the latter is not protected against phagocytosis.

Offensive-defensive Mechanism in Infections with Semiparasites.—

If now we turn our attention to the offensive-defensive mechanism which is thrown into operation in infections with the so-called semiparasites, of which the typhoid bacillus and the cholera vibrio are typical examples, we meet with still a different picture, which is fairly well defined also, although it has not been worked out in its details so thoroughly as we have seen it in anthrax. A great deal again depends upon the quantitative relations at the point of infection. If the infecting dose (of the cholera vibrio, for example, given intraperitoneally) is large, *e. g.*, several multiples of the quantity which will just produce infection, there is virtually no evidence of a defensive reaction. The organisms multiply from the start, or at least do not diminish in number even during the first few hours; there is no evidence of phagocytosis or of extracellular degeneration. Leukocytes indeed are relatively scant, while the abdominal cavity is filled with a serous exudate, in which the bacteria multiply as in an ordinary culture medium. The animal at the same time shows evident signs of being ill; the abdomen is tense and exceedingly tender, the hair is ruffled, the temperature drops, and death soon results.

From such a picture one would be led to conclude that the animal was devoid of all defensive means against the organism in question. This, however, would be erroneous, for on injecting another guinea-pig with a much smaller dose, *e. g.*, one-half the minimal infecting dose, which after all represents an enormous number of bacteria, the findings will be altogether different. If specimens of the peritoneal contents are removed at various intervals after the injection, it will be observed at a very early period that active bacteriolysis is already going on which may indeed be so extensive that after one hour the peritoneal cavity may have become microscopically free of organisms. But even if this does not result, the destruction of bacteria is in any event very considerable, and becomes complete through the introduction of a new factor, *viz.*, the appearance of

large numbers of leukocytes which are mainly of the polynuclear neutrophilic type. These dispose of the remaining organisms by phagocytosis, and the peritoneal cavity finally becomes sterile.

This means, in other words, that the animal which showed no evidence of a defensive reaction in the first experiment, actually had a very definite mechanism of this kind at its disposal, and the conclusion is, no doubt, justifiable that in the first instance the distribution of the normal bactericidal substances of the serum among the enormous number of bacteria (or its exhaustion by relatively few organisms) was insufficient to bring about any recognizable effect, and its renewed production, if, indeed, this occurred at all, was too small or delayed too long to cause any material retardation of the final outcome.

The appearance of the second line of defence, viz., the leukocytes, was evidently also delayed too long, if, indeed, we are permitted to speak of a delay at all under such conditions where there is evidence, both experimental and clinical, to show that in infections with overwhelming numbers of organisms the leukocytic mobilization may be arrested almost altogether.

If we compare the picture illustrated by the second experiment with what we have seen in the corresponding anthrax experiment there is a certain resemblance, for here as there the peritoneal cavity is virtually freed from bacteria soon after the primary invasion; but while in infections with the semiparasites or at least with organisms of the type of the typhoid and cholera bacilli, the organisms *remain* absent, or become so (unless too large a dose had been chosen) in anthrax there is invariably a second phase which is characterized by the return of the germs and their subsequent multiplication without further hindrance, even when a small dose has been injected.

Another point of difference also exists which is important, for whereas in the anthrax experiment the primary bactericidal effect was due to an associated phagocytic and aphagocytic activity of the leukocytes (in the presence of serum), the primary destruction of the cholera vibrios was essentially brought about by the normal bacteriolysins of the serum. Whether during the second phase of the cholera experiment, when the leukocytes appear, a leukin action also takes place, seems doubtful. If it occurs it certainly plays a relatively insignificant role. Then, again, while animalization (encapsulation) of the anthrax bacilli leads to successful resist-

ance against phagocytosis, the corresponding changes which take place in the cholera vibrio and the typhoid bacillus and which are represented by an hypertrophy of the ectoderm, do not lead to the same degree of protection.

Evidently, then, there is a marked difference in the character of the strife between the defensive forces of the guinea-pig and the two types of organisms. On the one hand, the anthrax bacillus gains the upper hand through its successful resistance to phagocytosis and the inhibitory effect of its aggressins upon the production of leukins, even though the appearance of the leukocytes at the point of infection is not seriously impeded. In infections with the semiparasites, on the other hand, the organisms conquer essentially through the negatively chemotactic effect of their aggressions upon the cells, which are thus kept at a distance and through the resistance of the animalized individuals to the ordinary bacteriolytic influences of the serum.

Between the two extremes which have been represented above, *i. e.*, the effect following the injection of large and of subinfectious doses of the cholera vibrio every possible gradation is possible and can actually be reproduced in the animal experiment. If thus the minimal infecting dose is injected there will usually be a primary bacteriolysis of considerable extent, which then gives way to a gradually developing increase in the number of the organisms. At first, as the leukocytes begin to appear, this proceeds slowly, but after a little while the organisms definitely secure the upper hand and coincidentally the further influx of cells is more or less completely arrested and the disease pursues its course to a fatal termination.

That the leukocytic insufficiency is really the deciding factor in the victory of the bacteria in such a case can be very well shown by previously injecting the animal with the leukocytes of a second one and then introducing the minimal dose of bacteria which in the untreated animal would invariably produce a fatal result. It will now be seen that the animal does not succumb, and if a series of corresponding experiments be carried out it can be demonstrated that a number of multiples of the originally just infecting dose must be injected in order to kill. Weil has shown very satisfactorily that this result is purely referable to the action of the leukocytes and not to any bacteriolysins that may be present, by previously rendering the latter inactive with so-called complement-binding

substances, when infection in the untreated animal may be brought about with subminimal infecting doses (as compared with a control animal), while in one that has been previously rendered hyperleukocytic this effect is not obtained.

Upon the basis of analytical studies such as those outlined in the foregoing pages, incomplete as they are, we can now distinguish three different types of infection (excluding those with the so-called necroparasites, in which a successful infection can scarcely be brought about under ordinary conditions). In the first, represented by the anthrax bacillus, the serum in itself is either inactive or shows but slight inhibitory qualities, while the combination of serum with leukocytes has strong antibacterial properties which can be completely overcome, however, through the aggressivity of the organism.

The second type is represented by various streptococci, staphylococci and certain vibrios (el Tor.), *i. e.*, organisms which stand very close to the group of the true parasites. In such infections the serum alone manifests but little bactericidal effect, while the antibacterial action of the serum, when combined with leukocytes, is strongly marked and can be only partially overcome by the aggressivity of the organism.

In the third type the serum alone, as well as in combination with leukocytes, shows marked antibacterial properties, the former by itself being sometimes sufficient to offset the aggressivity of the corresponding bacteria. The animalization of the organisms is here of little avail, as a protective measure against phagocytosis, while it is partially effective in the case of the serum. Most members of the typhoid and the vibrio group fall under this category.

CHAPTER VII

ANTIGENS AND ANTIBODIES

WE have seen in the foregoing chapters that the *normal* animal has a defensive mechanism at its disposal with which it may successfully meet a developing infection, with certain organisms at least, providing that the invading numbers are not too large. In laboratory parlance we express this by saying that successful resistance is possible, if the bacterial dose falls short of the minimal infecting amount, or if this should be exceeded, at least of the minimal fatal amount.¹

If now we compare the bacteriolytic titer of the serum of an animal that has received an injection of a subfatal dose with that of a normal control, or with that which the same animal showed before the injection, a remarkable increase will be noted which may be further raised by additional injections. Upon then examining the peritoneal contents of a normal animal that has received a minimal fatal dose and comparing the results with the findings in a second animal which has been previously injected with a subfatal dose and which now receives the same amount as the first, it will be noted that at a certain time the peritoneal fluid of the previously injected animal will have become sterile, while that of the untreated control is swarming with organisms; and, moreover, while the latter dies, the other recovers and thus shows itself, relatively at least, immune, using this term in the original sense of its meaning and synonymously with "resistant."

This immunity was evidently produced through the activity of the animal itself, and is hence appropriately spoken of as *active immunity* in contradistinction to *passive immunity*, which latter results when the immunity-bestowing substances that were actively produced in the one animal are artificially transferred to a second (normal) one. The possibility of such a transference can be readily

¹ These considerations apply essentially to infections with the so-called semi-parasites, exemplified by the cholera vibrio and the typhoid bacillus.

demonstrated by injecting a normal animal with a minimal fatal dose of the corresponding bacteria together with an appropriate quantity of serum obtained from an "immunized" animal. In such an event death does not result, because the animal has here been passively immunized by the serum of the immune animal, and now in turn develops an active immunity as the result of the introduction of the bacteria.

In a previous chapter we have seen that the bacteriolytic action of *normal* serum is referable to the associated activity of two substances, viz., the thermolabile complement and the thermostabile amboceptor. On studying a bacteriolytic *immune* serum in this direction, it may be shown that here also the destructive action upon the bacteria is dependent upon complement and corresponding amboceptor, and that its greater degree of activity as compared with normal serum is altogether owing to an increased content of the latter.

At a time when the antibacterial action of the normal blood serum was first discovered the question of the origin of the "alexins" was wrapped in complete obscurity. In view of the manner in which the production of the *immune* amboceptors takes place there can be no doubt that a direct connection exists between their appearance and the introduction of the corresponding bacteria, and upon injecting different animals with different species of bacteria we obtain evidence of a most remarkable specificity in the nature of the response, which one can well compare to the vibratory response which is called forth in tuning forks of different pitch by striking forks of corresponding pitch.

Further studies in this direction have shown that the appearance of such immune amboceptors takes place according to a fairly definite rule: immediately following the injection a period of latency can thus be observed which lasts for a few days and is then followed by a critical ascent of the curve leading to a maximal point from which there is in turn a corresponding drop which at first is fairly abrupt and later more gradual, and hence a slow return to previously existing conditions. As the same result is obtained after the injection of *dead* bacteria it is clear that the prolonged effect which follows the introduction of the organisms cannot be referable to possible variations in their number which one might otherwise imagine to be operative on different days and at different hours, nor can the

cessation in the formation of the amboceptors be explained on the basis of the gradual disappearance of the bacteria.

On the contrary, it is evident that a stimulus has been given which remains operative long after the primary impulse to amboceptor formation has ceased; to return to our simile, the second tuning fork still vibrates, though the first one which gave rise to its vibration has already become quiescent. The animal has coincidentally developed a resistance to the organisms in question which is far beyond its original value; it may indeed be absolute so that subsequent infection is altogether impossible. This resistance, moreover, in the case of some organisms at least, may be lasting, *e. g.*, the immunity which follows an attack of typhoid fever or of Asiatic cholera in man.

If the blood of a recently injected animal (using the typhoid bacillus for example) is further examined it will be found that, aside from the resultant bacteriolytic properties, it has developed still other characteristics which the serum of the untreated animal either did not possess at all, or if so, only to a slight extent. For it will be observed that such blood, even though freely diluted, has now the power of causing the arrest of motility and the clumping or agglutination of the corresponding organisms (*Widal reaction*), and this result, like the production of the bacteriolysins, is not dependent upon the introduction of living bacteria, but may be effected with dead organisms as well. If, further, analogous experiments are carried out with organisms like the diphtheria or the tetanus bacillus it will be observed that still other changes develop in the body of the infected animal and that bodies here appear in the blood serum which have the power of neutralizing the specific poisons formed by the organisms in question. Then, again, a curious reaction develops in animals which have been infected with the tubercle bacillus, for example, for on subsequent injection with certain derivatives of this organism (tuberculin) the animal responds with fever while the previously untreated control shows no reaction whatever.

Allergia.—These various responses in the reaction of the animal to the introduction of bacteria are now recognized as being merely a partial expression of a general biological law, to wit, that the animal organism invariably responds to the parenteral introduction of foreign cells (*i. e.*, the introduction of cells by other channels than through the gastro-intestinal canal, whether these be of animal

or vegetable nature), or of the products of foreign cells, insofar at least as they are of protein character, by the production of substances which in a general way tend to antagonize or even to destroy those which indirectly gave rise to their formation. For this altered behavior of the "treated" as compared with the "non-treated" animal, v. Pirquet has proposed the very appropriate and at the same time non-committal term *allergia* (ἄλλη ἔρρεα), which merely denotes a state of altered power of reaction on the part of the "treated" organism.

The reaction products which are formed in the body of the treated animal are conjointly spoken of as *antibodies*, and the substances whose introduction from without give rise to their formation are similarly termed *antigens* or *allergens*.

The discovery of these substances and their bearing upon the subject of immunity has opened up an enormous field for fruitful research, not only in the domain of medical science, but in that of general biology as well, and has already led to results which the boldest flight of the imagination would not have thought possible twenty-five years ago. The earliest and, in a manner, the most brilliant investigations in this direction we owe to the genius of Behring and his collaborators, Wernicke and Kitasato.

Antitoxins.—These investigators found that the serum of animals which had been rendered immune to the specific toxins of tetanus and diphtheria had acquired the power of neutralizing the harmful effect of those poisons, and Tizzoni and Catani introduced the term antitoxin to denote the substance to which this action is due. Here the way was shown for the first time along which it would be possible successfully to combat one of the most common and most dangerous diseases which has threatened the human race since time immemorial. Scarcely twenty-five years have now passed since Behring's announcement to the world (1890) that it is not only possible to protect the human being against infection with the diphtheria bacillus, but that the disease may be arrested even after it has gained a foothold—and all this through the injection of a relatively small amount of serum derived from a horse that has been previously treated with a culture of diphtheria bacilli or their specific toxin. How well Behring's discovery has served the human race is already a matter of history.

For a while hope ran high that it would only be a matter of time

before equally efficacious antitoxins would be discovered for the treatment of all the other bacterial infections to which both man and beast are prone, but this was soon doomed to disappointment. Why this should be is now fairly clear to us, since we have become familiar with the offensive mechanism through which the foreign organism seeks to maintain itself in the animal body, and through which the destruction of the host may even be accomplished. We have thus seen that both the diphtheria and the tetanus bacillus are organisms of the lowest grade of infectiousness which cannot possibly maintain themselves in normal tissues and are readily and rapidly destroyed through the activity of both serum and cells, but which kill nevertheless through the wonderful activity of their specific poisons. Against these the normal organism either possesses no antitoxin at all or such small amounts that a fatal end only too often occurs even though the infection, as such, has been or is being successfully combated. As a result of the infection, an attempt at antibody (antitoxin) formation is, of course, made (active immunization), but unfortunately the toxin may be able to produce its harmful effect before enough antitoxin is formed to neutralize its action. That under such circumstances the introduction of antitoxin from without (passive immunization) is the logical method of treatment, goes without saying.

In other infections the conditions are different. Unfortunately, the majority of organisms which are pathogenic for man are either not true toxin producers at all, or, if so, their infectiousness is of a much higher order, so that the mere introduction of an antitoxin, even though it were tuned to the corresponding toxin, so to speak, would not suffice to bring the disease resulting from the infection to a standstill. What is needed in such cases is something that will prevent the continuance of the infection, and that something can scarcely be of the nature of an antitoxin.

Aside from diphtheria and tetanus, there is actually only one organism, infection with which lends itself to antitoxin treatment, pure and simple, namely, the bacillus botulinus. Of the other pathogenic organisms the bacillus pyocyaneus, the staphylococcus, the typhoid, paratyphoid, and dysentery bacillus, the vibrio of cholera Asiatica and related organisms, the plague bacillus, and the bacillus of symptomatic anthrax are known or supposed to form true toxins even though to a limited extent only; but for the reasons just

indicated the corresponding antitoxic sera are of little avail in the treatment of the corresponding maladies.

Interesting from theoretical grounds is the fact that many other true toxins have been discovered which are not of bacterial origin. To this category belong certain snake poisons (venin), the phrynolysin found in the skin glands of toads (*Bombinator igneus*) and salamanders (*Sieboldia*), a poison obtained from special glands of certain fishes (*Trachinus*), the arachnolysin of various spiders (*Latrodectes* and *Epeira*), the poison of wasps and bees, the ichthyotoxin which is found in the serum of the eel, and possibly also the toxin producing fatigue, which, according to Weichardt, is formed in the muscles after severe exercise (kenotoxin). In addition to these, certain toxins produced by higher plants are recognized as possessing true antigenic properties, such as the ricin obtained from the seeds of the castor oil bean (*Ricinus communis*), the abrin of the jequirity bean (*Abrus precatorius*), the crotin of croton seeds (*Croton tiglium*), the robin obtained from the bark of the *Robinia pseudacacia*, and the phallin of the poisonous mushroom *Amanita phalloides*.

All these substances are characterized by their poisonous nature and the fact that their introduction into the animal organism, in suitable dosage, gives rise to the production of corresponding antitoxins which in turn have the power of neutralizing the toxic effect of the substances that gave rise to their formation.

Bacteriolysins.—Closely following upon the discovery of the antitoxins came the work of Pfeiffer and his pupils on the bacteriolysins (1894), viz., antibodies which result upon immunization (vaccination) with various bacteria and which possess the property of causing the dissolution of the corresponding organisms (*Pfeiffer's phenomenon*). Antibodies of this order are notably produced against certain vibrios, such as the vibrio of cholera Asiatica, the vibrio Metschnikoffi and related forms, against the typhoid and paratyphoid bacillus, the colon bacillus, the dysentery bacillus, the bacillus pyocyaneus, the influenza bacillus, and the bacillus of bubonic plague.

When these substances were first discovered it was hoped that the corresponding bacteriolytic sera would be found to possess curative properties analogous to those of the antitoxic sera, but it was soon ascertained that while they can prevent infection when they are introduced together with the organisms or shortly after, they are of little if any apparent avail in combating an already established

infection. Why this should be is not clear, unless we assume that the organisms have developed new characteristics, in consequence of which they are no longer open to attack by the bacteriolysins of the serum, and that the subsequent defence of the body must be carried on by other forces. For the correctness of this view there is some actual basis (see preceding chapter), but even so the last word on the use of the bacteriolytic sera has probably not yet been spoken.

But in any event the discovery of the bacteriolysins must be regarded as one of the greatest importance, as it has enabled us to gain a certain insight into the defensive mechanism of the animal body, which is most essential to further advance. Practically important is the fact that the action of the bacteriolytic immune amboceptors is specific and thus permits of a twofold diagnostic application. As the amboceptor content of the immunized animal is always higher than that of the normal control, a higher titer in reference to a given organism may be regarded as evidence of a preceding infection. Similarly one can use an immune serum for the purpose of identifying a given organism by comparing its action with that of a normal serum upon the organism in question, in the peritoneal cavity of a guinea-pig. Both methods are in actual use, the first for ascertaining whether or not an individual has recently passed through an attack of cholera, the other for establishing the identity of the corresponding organism after its isolation from the feces. (For a description of the method see Diagnostic Bacteriolytic Reactions.)

Agglutinins.—The next group of antibodies was discovered by Gruber and Durham (1896). These are termed agglutinins from the fact that the sera in question, when brought together with emulsions of the corresponding organisms, will cause the “clumping” or agglutination of the bacteria, and if these are normally motile, incidentally affect their loss of motility. As this property also is specific within certain limitations and the technique involved in its demonstration very simple, the principle has been extensively utilized for diagnostic purposes. As in the case of the bacteriolysins it may be applied both for the identification of a given organism and in search for the corresponding agglutinin. Under the name of the *Widal reaction* the test is now used the world over as one of the most important factors in the diagnosis of typhoid fever (see Agglutination Reaction).

The significance of the process of agglutination is not very clear.

That the life of the organism in itself has nothing to do with the production of the phenomenon is proved by the fact that the same result is brought about with dead cultures. On the other hand it can be shown that the process of agglutination does not lead to the destruction of the bacteria; these may, in fact, multiply in the agglutinated state. Under certain conditions they will then grow out in threads which are twisted upon themselves so as to form complicated skeins—a behavior which was first noted by Pfaundler and which is spoken of as Pfaundler's *Fadenreaktion* (*thread reaction*).

Gruber, Durham, Baumgarten a. o., at first looked upon the agglutinins as being identical with the bacteriolysins, the process of agglutination being interpreted as a stage preparatory to bacteriolysis. From this standpoint their formation could be viewed as evidence of a protective reaction on the part of the animal body. Subsequent investigations, however, have rendered this position untenable. Cholera immune serum thus loses its agglutinating properties after a certain length of time, even though its bacteriolytic power remains in full activity. Then, again, it has been observed that in typhoid fever the agglutinative and the bactericidal power of the patient's serum do not necessarily run a parallel course, but may actually diverge. Gengou further showed that the agglutinins do not dialyze through collodium, while the lysins do, and that the injection of sodium carbonate increases the bactericidal, but not the agglutinative power. While the agglutinins are thus unquestionably not identical with the bacteriolysins there are reasons for believing that they may after all not be antibodies *sui generis* (see Precipitins).

The most important organisms with which agglutinin formation has been successfully produced are the typhoid and paratyphoid (A and B), the cholera and dysentery bacillus, the bacillus lacti aërogenes, the diphtheria bacillus, the tubercle bacillus, the plague bacillus, the bacillus of glanders, the influenza bacillus, Friedländer's bacillus, the bacillus of tetanus and of rhinoscleroma, the pyocyaneus and proteus bacillus, the bacillus enteritidis, the cholera vibrio, the micrococcus melitensis, staphylococcus aureus, streptococcus pyogenes, the pneumococcus, and the meningococcus intracellularis.

Precipitins.—Shortly after the discovery of the agglutinating power of certain antisera, Kraus ascertained that such sera when brought together with the clear filtrates of the corresponding bouillon cultures

will cause the appearance of a turbidity which gradually collects at the bottom of the tube as a precipitate (1897). Further studies then showed that this peculiar behavior is owing to the presence of definite antibodies in the sera of the injected animals, and that such antibodies are formed whenever foreign albumins either of animal or vegetable origin are introduced through parenteral channels. From their precipitating properties these substances have been termed precipitins, while the corresponding antigen is termed *precipitinogen*.

Like the bacteriolysins, the precipitins have been shown to be specific in their action, within certain limitations at least, and the reaction has accordingly been used for the purpose of identifying the origin of various albumins. In the form of the *biological blood test* the principle is now generally utilized for the purpose of determining the origin of blood stains, and upon the same basis it has been possible to establish zoölogical relationship between different animals (see Precipitin Test).

Of special interest is the fact that a number of investigators are now inclined to regard the agglutinating properties of the various antisera merely as one form of expression of the more general precipitating qualities of the same sera, so that according to this conception the agglutinins as antibodies *sui generis* would have no existence. It is supposed that agglutination among cells corresponds exactly to agglutination among dissolved albuminous particles, which latter process leads to what we are accustomed to speak of as precipitation. This view is supported especially by Kraus, v. Pirquet, and Wassermann. These observers could show that bacterial filtrates are capable of binding agglutinin and that the filtrates in question must hence have contained agglutinable substances. This is well brought out if cultural filtrates are added to a corresponding agglutinating serum in sufficiently large quantity. Under such conditions the serum may lose its agglutinins entirely. If insufficient amounts of filtrate are used, on the other hand, the agglutinating titer remains unaltered, the difference in behavior being explained by the assumption that much smaller quantities of precipitin are required to cause agglutination than to bring about precipitation.

Cytolysins.—Further studies of the peculiar reaction of the animal body to the parenteral introduction of foreign cells and their derivatives then led to the discovery that antibodies of amboceptor type,

i. e., amboceptors of the nature of the bacteriolysins, are formed not only following the injection of bacteria, but also upon *immunization* with other cellular elements, using the term immunization in the more modern sense of the word, *viz.*, to express the throwing into action of the remarkable mechanism which results in the development of what we term *allergia*, and of which the formation of antibodies is the outcome (see above).

Collectively we now term all those antibodies of amboceptor type which are specifically directed against animal or vegetable cells, *cytolysins* or *cytotoxins*, and we designate the individual members of this order according to the cell against which their action is directed (*sc.*, according to the type of the corresponding antigen) and thus distinguish between *erythrocytolysins* (*hemolysins*), *leukocytolysins* (*leukolysins*), *epitheliolysins*, *spermatolysins*, *hepatolysins*, *neurolysins*, *nephrolysins*, etc. The *bacteriolysins* would thus merely represent a species of cytolysins.

The *demonstration* of some of these antibodies is a very simple matter as their action in reference to certain cells leads to alterations which are very manifest, while with others we rather surmise than are able to prove that a specific effect has been produced. It should be mentioned, moreover, that while we frequently speak of these bodies as lysins, a true dissolution of the entire cell does not necessarily take place, and it would really be more appropriate to use the synonymous term cytotoxin.

The first cytolysins of animal origin to be discovered were the *hemolysins* (1898). After Belfanti and Cortone had first shown that the blood of an animal of a given species *A* (horse), which had been previously injected (immunized) with the blood of an animal of a different species *B* (rabbit), becomes highly toxic for all representatives of species *B*, Bordet demonstrated that this result is accompanied by extensive destruction of the red cells in the animal *B*. He also showed that the same effect upon the red cells could be produced outside of the body. As the hemolyzing power of the serum *A* disappears after heating to 50° to 60° C. for about thirty minutes, but is restored upon the addition of fresh normal serum which is itself non-hemolytic, Bordet concluded that the hemolytic effect of the immune serum depended upon the joined action of two separate bodies, of which one is present in every *fresh* normal serum and is thermolabile, while the other, thermostabile constituent, is formed only

as the result of immunization, and is hence exclusively found in the immune serum.

Ehrlich and Morgenroth confirmed these findings and succeeded in demonstrating the presence of both components in the fresh serum of the immunized animal. These two substances, as we have already seen, are now generally spoken of as *amboceptor* (*immune body*, *Bordet's substance sensibilisatrice*) and *complement* (*alexin* of Buchner and Bordet). These discoveries were of fundamental importance, as they immediately led to the recognition that the bacteriolytic action of immune sera is similarly due to the coaction of two substances, of which the one also is present in fresh normal serum, while the other only appears as the result of immunization. A proper explanation was thus given for Pfeiffer's original observation, made in 1894, that inactivated bacteriolytic goat serum recovers its bacteriolytic action when introduced into the peritoneal cavity of a guinea-pig.

Metschnikoff and Bordet showed that the same result is obtained by mixing such inactivated serum with fresh peritoneal fluid *in vitro*, or by adding fresh serum or freshly defibrinated blood, the reason, of course, being that under the conditions of the experiment the inactivated immune serum finds the necessary complement both in the fresh peritoneal fluid and the fresh blood.

Bordet then showed that while naked eye observation of the process of hemolysis suggests that the red cells undergo dissolution, this is in reality not the case, but that the hemoglobin only undergoes dissolution into the outer medium, and that the stromata of the cells (the shadows of the red cells) can be separated by centrifugation, and demonstrated as such.

Similar observations were made regarding the action of the corresponding cytotoxic sera upon ciliated epithelial cells and spermatozoa. When such cells are introduced into the peritoneal cavity of an animal that has been correspondingly immunized the cells promptly lose their motility; but while the epithelial cells gradually undergo destruction the spermatozoa remain unchanged.

While the changes which are thus effected by cytotoxic sera in the case of red cells, ciliated epithelial cells and spermatozoa are very apparent, and while such cells, hence represent excellent test objects for the purpose of studying the nature and mode of action of the immune sera in question, the demonstration of a neurotoxic, hepato-

toxic, or nephrotoxic influence is a more difficult task. Delezenne, it is true, claims to have obtained hepatotoxic sera by injecting suitable animals with dog's liver, and states that such sera will produce specific impairment of the liver function in dogs, as evidenced by diminished elimination of urea, by increased excretion of ammonia, and the appearance of leucin and tyrosin in the urine, and that digestive glucosuria may also develop when the animal receives an abundant supply of sugar; in some instances death even occurred. The same writer further claims to have obtained a neurotoxic serum which would produce vacuole formation and chromatolysis in ganglionic cells. Lindemann and Nefedieff further announced that with a nephrotoxic serum they could bring about the development of albuminuria, uremia, and death, and that post mortem there was widespread degeneration of the epithelial cells of the convoluted tubules.

Isocytolysins.—Observations such as these naturally raised the question whether cytotoxin production only occurs when cells from a given animal are injected into an animal of an alien species, *i. e.*, whether *heterocytotoxin* (sive *heterolysin*) formation only is possible, or whether something analogous does not occur when cells from one animal are injected into another one of the same species. Ehrlich and Morgenroth could show that the production of such *isocytotoxins* (sive *isolysins*) can actually occur, for on injecting goats with goat blood he found that the serum of the injected animals had become hemolytic for goat corpuscles. Metschnikoff similarly found that an isospermatoxin is formed when a guinea-pig is injected with spermatozoa from another guinea-pig, as is evidenced by the fact that such a serum will rapidly immobilize the spermatozoa. This was first shown *in vitro*, but subsequently also established for the living animal, for on injecting male mice with a corresponding serum they were rendered sterile and coincidentally the remarkable observation was made that the semen of such animals had lost its antigenetic properties, viz., that upon injection into other animals it had lost the power of calling forth a corresponding formation of spermatotoxin.

Auto-antibodies.—Further observation along these lines then led to the important discovery that iso-antibody formation not only is possible, but that *auto-antibodies*, viz., antibodies against the cells of the same animal which furnished the cells, can also be

produced. The demonstration of this possibility is, of course, most important from the standpoint of animal pathology, for it raises the question whether some of the symptoms occurring in diseases in which active cellular destruction is known to occur, or even some of the pathological lesions, may not be the outcome of the formation of *autocytotoxins*. Observations in this direction are as yet too meager to warrant any far-reaching conclusions. I would merely call to mind the now well-established fact that the serum in various pathological conditions has been shown to be hemolytic for the red cells of other individuals, and while there is evidence to show that the cells of the same individual (*i. e.*, the one furnishing the serum) are more resistant, the thought naturally arises, whether the anemia which is so frequent in the very diseases, in which auto-hemolysins have been demonstrated, viz., syphilis, tuberculosis, and cancer, may not in a measure be due to the action of such antibodies.

It has been argued that if such antibody formation actually did take place the harm done would be progressive, unless indeed an anti-autocytotoxin formation in turn were to occur. The production of such bodies also has actually been demonstrated by a number of observers (Bordet, Müller, Ehrlich, and Morgenroth), and it has been shown, moreover, that these substances are of the nature of *anti-amboceptors*. Of their role in the animal organism, however, as well as that of the auto-antibodies themselves, our knowledge is still most imperfect and a discussion of the various possibilities would at present amount to little more than a philosophical discourse.

Immune Opsonins and Bacteriotropins.—Further studies have shown that in addition to the various types of antibodies which we have considered so far there are still others.

We have had occasion to point out in a previous chapter that in the course of various infections, substances appear in the blood which prepare the corresponding organisms for phagocytosis, but which differ from the normal opsonins in their greater thermostability; these bodies have been designated as immune opsonins or bacteriotropins. Bodies of this character have been demonstrated following immunization with the streptococcus, pneumococcus, staphylococcus, meningococcus, the cholera vibrio, the typhoid and paratyphoid bacillus, the dysentery bacillus, the tubercle bacillus, the plague bacillus, and the anthrax bacillus. Of their mode of action nothing

definite is known. Neufeld inclines to the belief that they produce some alteration in the physicochemical status of the cell, in virtue of which some constituent of the cell body is transformed into a soluble modification which now surrounds the organism with a delicate layer and serves to attract the leukocytes. As I have already pointed out, the tropins can act independently of the presence of complement and are thus of simpler structure than the opsonins proper—both those occurring in normal, as well as those found in immune serum. The quantity of these substances which may occur in immune sera is sometimes very considerable and much greater than that found in normal blood; for whereas the latter can scarcely be diluted more than fifty times before it loses its specific action, immune serum may at times still call forth phagocytosis when diluted a thousandfold.

Antiferments.—Another group of antibodies are the so-called anti-ferments which are specifically directed against the corresponding ferments. Substances of this order have been observed in the blood serum after immunization with rennin, pepsin, trypsin, tyrosinase, thrombin, urease, lactase, lipase, laccase, etc., the corresponding antiferments being accordingly designated as antitrypsin, antipepsin antirennin, etc.

The discovery of bodies of this order is especially interesting as it throws some light on the vexed question: Why do the various cells of the body not digest themselves? In former years when the digestive ferments of the stomach and pancreas were the only ones known to occur in the animal body, it was a source of wonder why the corresponding digestive juices did not digest the organs in which they were produced. At the present time when we know that proteolytic ferments are present in every cell we may well wonder why the whole body does not digest itself. If leukocytes are removed from the body and suspended in saline or Ringer's fluid, self-digestion begins after a relatively short time and leads to the complete destruction of the cells, even though the access of bacteria be prevented. If, on the other hand, the cells are suspended in normal serum, autodigestion does not occur, and if such serum be tested against a solution of trypsin it can be shown that it possesses marked antitryptic properties. The discovery, then, that the antitryptic content of the blood can be materially increased by immunization with trypsin, suggests the possibility, at least, that, even normally,

antiferment production occurs in the animal body and renders the conclusion not unwarrantable that these antiferments are essential to the life of the whole organism.

Antilipoids.—Still more recent studies have brought to light another interesting class of antibodies which are not “tuned” to any particular cell, so far as our present knowledge goes, but which have the power of reacting with substances belonging to the group of lipoids. Such antibodies have been discovered in patients suffering from syphilis, yaws, frambesia, trypanosomiasis, cancer, leprosy, etc., and may, for the present, be spoken of as antilipoids. Their reaction with the corresponding lipoids is characterized by the fact that if complement be present at the time this is fixed to a greater or less degree, so that upon the subsequent addition to the mixture of lipid, antilipoid, and complement, of washed red corpuscles and a suitable hemolytic amboceptor, hemolysis either does not occur at all, or is more or less impeded. This remarkable phenomenon is the basis of the so-called *Wassermann test* for syphilis, and as such constitutes one of the most important discoveries of medicine (see Wassermann reaction).

Albuminolysins.—I have pointed out above that upon injecting albumins, either of vegetable or animal origin, into an animal of an alien species, antibodies are formed which are known as precipitins, and are characterized by their ability to form a precipitate, when brought together with the corresponding antigenic substances. The allergic state which develops as the result of the injection of foreign albumins can manifest itself in still other ways, however. If a guinea-pig is thus injected with normal horse serum, for example, and an interval of from ten to thirteen days is allowed to elapse, it will be noted that the second injection is followed by most alarming symptoms—intense dyspnea and marked drop of temperature (*Theobald Smith phenomenon*)—which frequently end in death. Corresponding symptoms occur in other animals and may follow the introduction of almost any foreign albumin by parenteral channels. Evidently the first injection sensitizes the animal to subsequent injections and the thought naturally suggested itself that here also antibodies may play a role. What these antibodies are is still a matter of surmise. Some investigators have attempted to identify them with the precipitins, while others look upon them as a peculiar type of lysins, which we may accordingly term albuminolysins,

and suppose that during the interaction between the lysin and its antigen, at the time of the second injection, highly poisonous intermediary products are formed to which the peculiar symptoms are in turn due.

Anaphylaxis.—As the injected animal has evidently become more sensitive to the action of the foreign albumin than it was before the first injection, which usually does not give rise to any serious symptoms, Richet suggested the term *anaphylaxis* to express this condition of hypersusceptibility, in contradistinction to prophylaxis, diminished susceptibility or immunity, in the older sense of the word. This term has now been generally accepted, and the more or less threatening symptoms which follow the second injection are accordingly spoken of as the *anaphylactic shock*. French writers, more particularly, refer these symptoms to a special anaphylactic reaction product which they term *anaphylactin*. v. Pirquet, as we have already seen, has introduced the non-committed term *allergia* to denote the changed mode of reaction on the part of the injected animal (no matter what antigen has been used) and speaks of the antigenic substances as the *allergens* and the reaction products as the corresponding *ergins*. According to his ideas, anaphylaxis is thus merely one form in which the general *allergia* can express itself. In a subsequent chapter we shall have occasion to deal with this problem in greater detail, and we hope to show that the antibodies which are especially involved in the anaphylactic reaction, play an important role in the symptomatology of many diseases.

The brief survey of the manner in which the animal body responds to the parenteral introduction of foreign cells and cell derivatives, which has just been given, imperfect and condensed though it be, is probably sufficient to show that a field of work has been opened up which offers a most alluring perspective to the investigator, both in medicine and general biology. During the few years that it has been tilled, the returns have already been wonderful in their diversity and value, and we have every reason to suppose that a great deal of the future progress of medicine will lie in this direction. We have already a host of experimental facts which only await their proper interpretation, before they will become important stepping stones toward still more important findings. Among the many able investigators who are closely associated with progress along these lines, one

stands out prominently above all others, because he has furnished us with a working hypothesis which satisfactorily explains many observations that have been made in this field, and because its study has opened up new avenues of research along which much fruitful work has already been accomplished. That man is Paul Ehrlich, and in the subsequent chapter we shall endeavor to show that on the basis of his now world-famous *side-chain theory* the formation of the many groups of antibodies with which we have already become acquainted can be explained and their specific properties accounted for.

CHAPTER VIII

THE SIDE-CHAIN THEORY

WHEN it was discovered that the injection of the serum of an animal that had been rendered immune to diphtheria could protect another animal against infection with the corresponding organism, and could even arrest the disease after this had actually developed, the question naturally arose how this remarkable effect could be explained. Different possibilities, of course, suggested themselves.

Roux and Buchner at first expressed the opinion that the antitoxin produced its effect by acting upon the cells of the body in such a manner as to increase their resistance, or to diminish their susceptibility to the corresponding toxin. In other words, they imagined that the antitoxin called forth a rapid immunization of the cell. This view was abandoned when it could be shown that the addition of antitoxic serum to a toxin outside of the body is capable of preventing the effect of the latter, when the mixture is subsequently injected into the body.

Ehrlich first demonstrated this with the blood of mice which had been immunized against the vegetable toxin ricin. He found that several multiples of the minimal fatal dose of this substance could be injected into animals, without producing any toxic symptoms, if an appropriate amount of blood from a correspondingly immunized animal (*antiricin*) had previously been added to the ricin solution. Fraser similarly found that the antitoxin directed against snake poison (*antivenin*) acted much more energetically, if it was first mixed with the corresponding toxin (*venin*) outside of the body, than when its injection immediately followed or preceded that of the toxin. Analogous experiments with toxic eel serum, croton, and the hemolytic component of the tetanus toxin (*tetanolyisin*) led to similar results.

These observations also rendered untenable the view that the antitoxin only acquires active properties as the result of a ferment-like transformation of the substance on the part of the body cells.

Evidently the *antitoxin acts directly upon the toxin*, and in interpreting the findings *in vitro*, different possibilities again arise. It is thus conceivable that the antitoxin may destroy the toxin. This view, however, has also been disproved by a number of observations. Buchner thus showed that a mixture of tetanus toxin and antitoxin which was "neutral" for mice was still toxic for guinea-pigs. Roux and Calmette further ascertained that a mixture of snake venom and antivenin which was non-toxic for a given animal, became toxic again on heating. This would evidently be out of the question if the non-toxicity of the mixture had been owing to a destruction of the toxin by the antitoxin. Martin and Cherry then pointed out that this result is obtained only if not too long an interval has elapsed after bringing toxin and antitoxin together, and that a restitution of the toxic effect is no longer possible if a certain time limit has been passed. They could show, as a matter of fact, that toxin and antitoxin do not interact instantaneously, and that at first toxin and antitoxin coexist in the free state, the velocity of reaction depending very largely upon the concentration of the two solutions and the temperature.

In the light of these findings Roux and Calmette's original observations do not disprove the idea that the antitoxin destroys the toxin. That this actually does not occur was, however, conclusively shown by Morgenroth in Ehrlich's laboratory; for on treating a mixture of Cobra toxin and antitoxin that had been kept for more than a week, *i. e.*, for a period of time that was more than sufficient completely to destroy any toxic effect by the antitoxin, with dilute acids, and on then heating the acid mixture for some time at 100° C. the original quantity of toxin was again obtained in its entirety. *A destruction of the toxin by the antitoxin had thus been satisfactorily disproved.*

Evidence such as this is, of course, strongly suggestive that the inactivation of the toxin by the antitoxin is due to the occurrence of a chemical interaction between the two, and that the specific effect of the toxin disappears because the substance is chemically bound by the antitoxin. This view, which was first expressed by Ehrlich, is the one now generally held and forms the basis of our modern conception of the production of antibodies and their specific effect.

Further studies have shown, as a matter of fact, that the same principle applies to the interaction between other antigens and their

antibodies. If washed red corpuscles are thus brought together with a corresponding hemolytic amboceptor and are incubated at body temperature, the amboceptor is anchored to the corpuscles and can no longer be removed by washing. That an interaction has actually taken place may be shown by adding fresh complement, when hemolysis will promptly occur. Analogous results are obtained with the bacteriolytic amboceptors, agglutinins, and precipitins, and as in the case of the antitoxins it may here also be shown that no destruction of the antigen takes place. If milk and a corresponding precipitin (lactoserum) are thus brought together, a precipitate of antibody casein is formed, and if this is boiled, after careful washing in normal salt solution, the precipitate dissolves, and in the resulting solution unchanged casein can be demonstrated, which may in turn be precipitated by the addition of a new portion of antiserum. The precipitin can similarly be recovered by treating the precipitate with $\frac{n}{100}$ sodium hydrate or sulphuric acid.

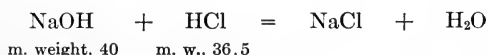
While a destructive action on the part of the antibody upon the antigen can thus be excluded, the latter may be destroyed secondarily in consequence of the activity of some additional factor. This actually takes place when complement acts upon cells that have been brought together with corresponding cytotoxic (lytic) amboceptors. The term lysis, of course, suggests that it is the amboceptor that is lytic, but it should not be forgotten that the lytic effect only occurs when complement is present, that the latter really is the lytic agent, and that the amboceptor itself produces no appreciable effect upon the antigenic cells.

Chemical Nature of Antigen-antibody Interaction.—The assumption that the interaction between antigens and antibodies is of a chemical nature carries with it the inference that the reacting substances must combine with one another in certain definite proportions or multiples thereof, viz., that if one unit of antitoxin will neutralize one unit of toxin, ten units of the one should combine with ten of the other, twenty with twenty, etc. The investigation of this particular side of the problem has led to a great deal of controversy arising from erroneous interpretations of various observations, owing to imperfections in technique, lack of knowledge of detail, etc., and has consequently been productive of an enormous amount of labor, for much of which we are indebted to Ehrlich and his school.

From the very nature of things the interrelation between toxins and antitoxins has received the greatest amount of attention, for here we are dealing with substances which react in solution and whose behavior is thus more readily open to investigation and interpretation than in the case of those phenomena which occur between highly complex components, such as animal and vegetable cells and their antibodies. It may be said in advance, however, that notwithstanding many observations which at first tended to suggest that the character of the interaction between toxins and antitoxins was of a different nature than that between the other antibodies and their antigens, more detailed investigations have shown that this difference is more in appearance than in fact.

A number of modern investigators have attempted to explain the laws which have been found to govern the action of antibodies upon their antigens upon a purely physical basis, but, although many observations may be interpreted as supporting their view, one factor is not explained upon these grounds, and that is the remarkable specificity of the antibodies. Ehrlich's side-chain theory, on the other hand, which rests upon a purely chemical interpretation of the phenomena of antigen antibody interaction, satisfactorily accounts for this, so that, even though we admit the validity of the physical theory in the explanation of certain phenomena we must still adhere to the chemical side. All the facts which have been observed when toxins and antitoxins interact can certainly be explained upon chemical grounds. In the case of the other antigens and their antibodies, we may admit that physical processes *may* play a role, but in addition to these, chemical action unquestionably also takes place. A somewhat more detailed account of certain studies along these lines will serve to bring out some of the difficulties which have been encountered.

As I have pointed out, if we conceive that toxin and antitoxin unite with one another chemically, then we would expect that definite quantities of the one or multiples thereof would unite with corresponding quantities or multiples of the other. To use a common example—if 40 parts by weight of sodium hydrate unite with 36.5 parts by weight of hydrochloric acid, according to the equation:



then 2×40 parts of NaOH will unite with 2×36.5 parts of HCl, and 3×40 NaOH with 3×36.5 HCl, etc.

When this point was first investigated with toxin-antitoxin mixtures it was found that starting with an apparently neutral mixture the injection of several multiples of this proved highly toxic; in other words, whereas the original mixture was apparently perfectly innocuous, a fatal result was obtained if from two to five times as much toxin was used, and this treated with corresponding multiples of antitoxin. Upon first consideration such a result would seem entirely contradictory to the idea that the toxin and antitoxin neutralize one another in a chemical sense. Further investigation, however, has shown that the contradiction is only apparent, and that the law of multiples does hold good for the toxin-antitoxin interaction, but that it is absolutely essential in such experiments to neutralize the original mixture with such exactness that not even a minimal fraction of toxin is present in excess of the antitoxin. If this is not the case, one can readily conceive that even though the original mixture were non-fatal, several multiples thereof might very readily be so. It is hence imperative that the original mixture should be so standardized that its injection does not cause the slightest symptom of disease. If this is carefully done then it will be found that the law of multiples actually does hold good, and this law, as a matter of fact, forms the basis of Behring and Ehrlich's method of standardizing the diphtheria antitoxin of the market, in which the unavoidable source of error does not amount to more than from 0.1 to 1 per cent. (see Preparation of Diphtheria Antitoxin).

If, now, we come to apply the law of multiples to the study of the other antibodies, we find that the possible sources of error in the concrete interpretation of the actual findings are still greater, and it may not be out of place to refer in some detail to some of the difficulties which have been here encountered and the manner in which they have been met. We may say in advance, however, that no observations have been made which would tend to exclude the interaction between these antigens and their antibodies from the law of multiples, as it has been established for toxin-antitoxin mixtures.

Starting with 1 c.c. of an emulsion of an agar slant culture of a given organism in 15 c.c. of normal salt solution, and treating this with an equal volume of an agglutinating serum in varying dilutions,

Eisenberg and Volk term that quantity of serum an agglutinin unit, which will bring about *partial* agglutination of the contained organisms in twenty-four hours. If, then, constant quantities of the bacterial emulsion (*e. g.*, 1 c.c.) are treated with an increasing number of agglutinin units it will be observed that the bacteria have the power of absorbing an enormous excess of agglutinin beyond the amount that is actually required to produce agglutination. If, moreover, the number of units that is actually absorbed is compared with the number added, the interesting fact develops that *with increasing concentration of the agglutinins the absolute absorption by the bacteria rises, while the absorption coefficient, i. e., the ratio between the number of units added and the amount absorbed, falls*. This is well shown in the accompanying table which is taken from Eisenberg and Volk.

ANTITYPHOID SERUM, ZOROASTER III. AGGLUTINATION VALUE = 45,000 UNITS

Serum dilution.	Agglutinin units added.	Agglutinin units absorbed.	Coefficient of absorption.
1 to 20000	2	2	1.0
1 to 2000	22	22	1.0
1 to 1000	45	45	1.0
1 to 600	75	75	1.0
1 to 500	90	89	0.99
1 to 200	225	210	0.93
1 to 100	450	400	0.88
1 to 20	2250	1650	0.73
1 to 4	11250	6750	0.60
1 to 2	22500	12500	0.56
1 to 1	45000	22500	0.50

The question then arises how to explain the apparent paradox that the same quantity of bacteria which can only absorb 12,500 units out of 22,500 that have been offered can actually absorb 22,500 when brought in contact with a proportionately larger amount. Upon first consideration the thought of a chemical union between agglutinin and agglutinable substance would seem to be out of the question. Various explanations, however, have been offered, any one of which would show that the paradox is in reality only apparent. As will be seen later on there are reasons for supposing that an agglutinating serum may contain not only one single agglutinin, but a number of agglutinins which correspond to the presence of an equal number of agglutinable substances (agglutinogens) in the body

of the bacillus; as experience, moreover, has shown that different antigens, even though closely related, may differ very considerably in their antibody forming power, we may assume that the number of agglutinable molecules in a unit of bacterial emulsion is different from that of the various agglutinins in a unit of the corresponding serum.

Supposing, now, that in the former there were present 100 molecules of the agglutinable substance a , 50 of the agglutinable substance b , and 20 of c , while in the antiserum there were present for each unit 100 molecules of agglutinin A , 20 of B , and 2 of C , the 100 a 's would then unite with the 100 A 's, 20 b 's with the 20 B 's, and the 2 c 's with the 2 C 's. There would then be remaining 30 unsatisfied molecules of b and 18 of c . If, therefore, a second unit of agglutinating serum were now added, 20 of the remaining b 's would take up the 20 newly added B 's and 2 of the remaining 18 c 's the 2 new portions of C . There would now remain 10 molecules of b and 16 of c , while the 100 A 's from the second agglutinating unit would be left over. This would represent exactly what we see in the table above, viz., that even though agglutinins in excess be present the bacterial emulsion can still take up more agglutinin if more is added.

The reason for this apparent paradox is now, of course, self-evident, and lies in the fact that we have been mentally in the habit of ascribing the agglutinating properties of a given serum to a single substance; whereas there is good evidence to show that this is not necessarily the case, that on the contrary the agglutinating effect may be due to a number of so-called *partial agglutinins*, to which a similar number of agglutinogens correspond, and that the quantities present in the serum do not tally with those in the bacterial emulsion. The *Eisenberg phenomenon* is thus merely the expression of the coexistence in the mixture of free antigen on the one hand and free antibody on the other, the antigen being in excess merely because not enough antibody has been added.

Such a coexistence may, however, also be explained in still other ways, showing that there is really nothing unusual in the phenomenon. According to the Guldberg-Waage *law of mass action* the quantity of two chemically reacting substances a and b and their product c which may be found at any one time in coexistence, depends upon a certain constant k , which varies only with the nature of the

reacting substances and the temperature. Chemical equilibrium will result in accordance with the equation—

$$\frac{(Ca)^n \cdot (Cb)^m}{(Cc)^o} = k$$

in which a and b represent the reacting substances, c their product, Cc , the concentration, and n , m , and o the respective number of molecules. If we conceive that only one molecule of the reacting substance enters into play the equation may be simplified so as to read—

$$\frac{Ca \cdot Cb}{Cc} = k$$

As k is constant it will be seen that by increasing the concentration of either a or b the concentration of the product also must be increased. If, now, we conceive k to be infinitesimally small or even equal to zero, then the concentration of either a or b or of both must be zero, since c itself cannot be zero. In this case we would come to an end reaction where both a and b would be completely used up and only the product remain. If, on the other hand, k is infinitely large then the concentration of c must be correspondingly small, and if $k = \infty$ then c must equal 0, which means that no union whatever occurs between a and b . Between these two extremes, viz., $k = 0$ and $k = \infty$, an infinite number of variations is, of course, possible, and it is thus readily conceivable that k in the case of the agglutinin-agglutinable substance reaction may be of such a value that a partial reaction only is possible between the two. This, of course, is what we see in the Eisenberg phenomenon, and accepting this explanation we would have additional proof that the reaction between antigen and antibody is actually of a chemical character.

Still another explanation is possible on the basis of the so-called *law of distribution*. This is based upon the following considerations: If a given substance a is soluble in two solvents A and B and if the substance in question is brought together with A and B simultaneously a certain amount will be dissolved in A and a certain amount in B . The ratio between the two amounts is a constant which varies only with the nature of the substance in question and the temperature, but which is uninfluenced by the initial concentration. From a study of the figures given by Eisenberg (see above), Arrhenius

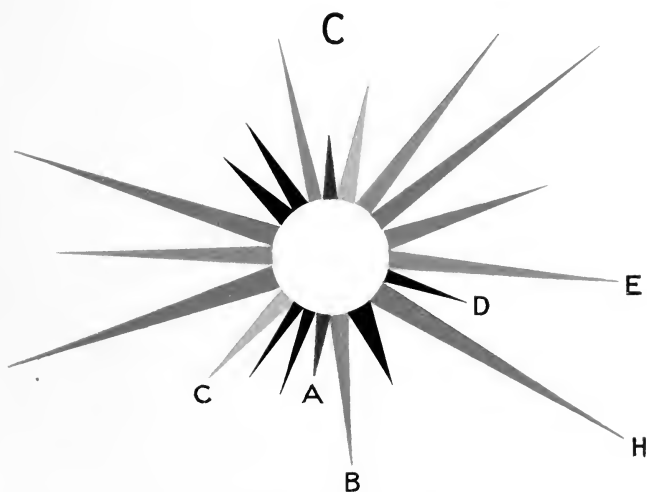
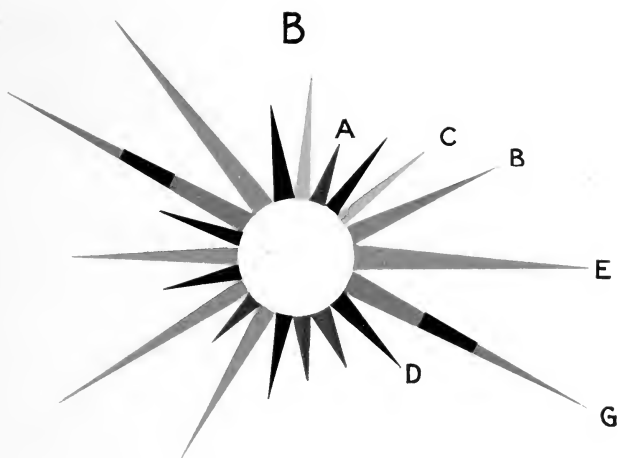
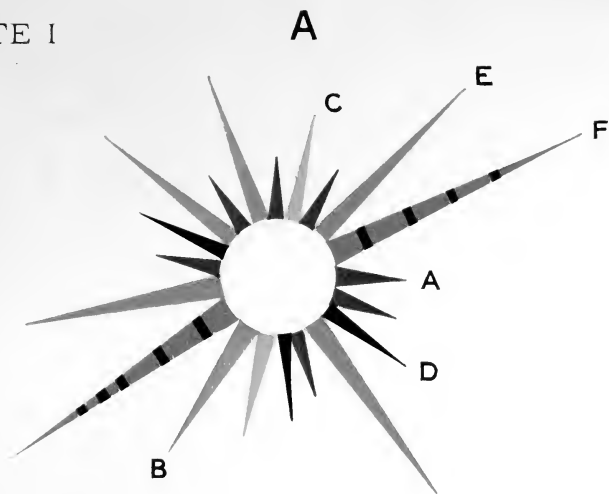
concluded that the peculiar relationship between the amount of agglutinin present in the free state and that taken up by the bacteria could be readily accounted for on the basis of the law of distribution, and he developed for this particular case the equation

$$\frac{(\text{Quantity of bound agglutinin})^3}{(\text{Quantity of free agglutinin})^2} = k \text{ (constant)}$$

The figures actually observed in the experiment and those theoretically expected are, indeed, so nearly alike that it would seem unnecessary to seek for any further explanation of the Eisenberg phenomenon (see accompanying table). But even though we admit that the *manifest* antigen-antibody reaction can thus be satisfactorily accounted for on the basis of purely physical absorption, this in itself does not preclude the possibility of a subsequent chemical interaction between the two substances.

No. of agglutinin units added.	No. of units absorbed according to observation.	No. of units absorbed according to calculation.
2	2	1.98
20	20	19.3
40	40	37.9
200	180	180.3
400	340	347.1
2000	1500	1522.0
10000	6500	6110.0
20000	11000	10840.0

Formation of Antibodies.—If now we pass on to a consideration of the question how the introduction into the body of a given antigen can lead to the formation of corresponding antibodies, we do so upon the basis that, even though physical laws may be operative during the interaction between the two classes of substances, actual chemical union must invariably occur. This, indeed, constitutes the very key-stone of Ehrlich's *side-chain theory*, a study of which must now engage our attention. According to Ehrlich's doctrine, we must look upon every cell, stereochemically speaking, as being composed of a central molecular complex upon the integrity of which the life and activity of the cell depends, and of a variable number of subsidiary molecular groups which serve the purely vegetative functions of the cell. The former Ehrlich appropriately designates as the "*Leistungskern*," or functional nucleus of the cell, and the latter as the so-called "*Seitenketten*," or *side chains*.



Diagrammatic Representation of the Functional Nucleus of Three Different Types of Cells and the Different Quantitative Relations of the Various Side Chains.

- A. $a=35\%$, $b=15\%$, $c=10\%$, $d=15\%$, $e=15\%$, $f=10\%$, $g=0$, $h=0$
- B. $a=20\%$, $b=30\%$, $c=10\%$, $d=15\%$, $e=15\%$, $f=0$, $g=10\%$, $h=0$
- C. $a=10\%$, $b=30\%$, $c=10\%$, $d=15\%$, $e=15\%$, $f=0$, $g=0$, $h=20\%$

Through the side chains the nutrition of the central nucleus may be conceived to be regulated, and as differences in function no doubt presuppose certain underlying differences in chemical composition, and hence differing chemical affinities for those substances which constitute the foodstuffs of the cell, we may well imagine that not all the side chains of a given cell (through which the nutritional processes must take place) are chemically alike, and that certain side chains are peculiar to a certain cell type, while others are common to all cells. This conception of the chemical structure of the cell may be diagrammatically expressed by representing the functional centre as a sphere, from which variously colored rays—the side chains, emanate, and the difference between a nerve cell, for example, and a connective-tissue cell or muscle cell could be expressed by the presence of a different percentage of rays of a common color and the additional presence of special rays of differing tints. This I have attempted to illustrate in the accompanying illustration (Plate I).

It will be seen that all three types of cells have *a*, *b*, *c*, *d*, and *e* rays in common, but that these are present in different percentage proportions, and that the cells differ from one another not only in this respect, but also in the exclusive presence of *f* rays in the one, of *g* rays in the other, and of *h* rays in the third. Ehrlich further conceives that a given foodstuff can only be utilized by a given cell, if it possesses atomic groups which are capable of combining with corresponding groups of the side chains. As the latter are not all alike in their chemical structure, it is reasonable to suppose that a definite relationship must exist between their combining groups and the combining groups of the foodstuffs, such that certain food molecules only will be capable of uniting with certain side chains.

To use the frequently quoted simile which Emil Fischer first applied to the specific action of ferments, we may say that the combining group of the food molecule must fit a corresponding group of the side chain like a complex key fits its special lock. To revert to our diagram we may express this by assuming that only a black food molecule can combine with a black side chain, only a green one with one of its own color, etc.

All those side chains which are capable of combining with chemical bodies in general, Ehrlich designates collectively as *receptors*, or *chemoreceptors*, while those which react with *foodstuffs* more or less

exclusively, and which accordingly serve the nutrition of the cell, are appropriately termed *nutriceptors*.

Under ordinary conditions of cell life, we can well imagine that the cell receptors will have occasion to react only with actual food-stuffs. But we can also conceive that under abnormal conditions, as in the various infections, substances may be brought to the cell which accidentally possess an atomic group that is identical in structure with the combining or *haptophoric group*, as it is termed, of the usual food molecule to which the special receptor is "tuned."

To use a homely simile, we may say that while ordinarily only the rightful owner of a house can unlock its doors, the possibility exists that a burglar with a master key could similarly gain entrance; and to carry the simile farther; while the entrance of the house-owner would not be attended by any undesirable consequences, the result might be quite different in the case of the burglar. To return to actual conditions we can readily see that the existence of such a combining group on the part of a toxin molecule, for example, might actually be fatal to the life of the cell, supposing, of course, that the *Leistungskern* itself could be injured by the toxin. As a matter of fact, this is exactly what Ehrlich supposes to occur in such infections as diphtheria, tetanus, and botulismus. He conceived that the toxic molecule in question must have two distinct molecular groups, one of which—the *haptophoric group*—anchors the toxin to the cell receptor, while the other—the *toxophoric group*—is the actual bearer of its toxic properties.

If now we imagine that the number of toxin molecules which have thus gained access to the cell, in virtue of the identity in the structure of its *haptophoric group* with that of the usual food molecule is not sufficiently large to cause its destruction, the cell would nevertheless suffer to a greater or less extent owing to the occupation of important *nutriceptors* by material that possesses no food value, unless indeed it succeeds in freeing itself of its undesirable encumbrance. When the *nutriceptors* combine with ordinary food molecules we may imagine that this union is not permanent, but that the food molecules are used up chemically to supply the needs of the cell. Coincidentally the corresponding receptors are again liberated and placed in a position where they can combine with a new set of food molecules, and so on. If the toxin molecule, on the other hand,

cannot be destroyed in this manner the cell must use some other method to rid itself of the offending material.

According to Ehrlich's concept, it accomplishes this by casting off the receptor together with the anchored toxin molecule. The resulting defect in its structure, the cell then makes up by a production of new receptors of the same kind. In accordance with Weigert's law of regeneration this new production, however, takes place in excess of the actual requirements, a condition of affairs which the cell meets by throwing off the unnecessary number of receptors as such. These cast-off receptors will, of course, have the same combining groups as the sessile ones, which had originally anchored the toxin molecule, and it stands to reason that if the toxin molecule and the corresponding free receptor are brought together either within or outside of the body the two will unite, the result being indicated by absence of toxicity on the part of the mixture. As this is exactly what happens when toxin and the serum of a correspondingly immunized animal are brought together, Ehrlich very properly concludes that the antitoxic properties of an immune serum are due to the presence of free receptors which are "tuned" to the toxin in question; in other words, that the antitoxin is not newly formed in the body, but is identical with those receptors of the cell which render the attack of the toxin upon the cell possible.

While this conception of the nature, production, and mode of action of the antitoxins originally had reference to these only, subsequent observations led Ehrlich to extend his theory to the other antibodies as well. But in accordance with the facts observed it is necessary to assume that the structure of the other antibodies, viz., those receptors which enter into relationship with such antigens as the agglutinable substance of bacteria, the precipitable complex of albumins and those cellular constituents which give rise to lysin formation, must be different from that of the antitoxic receptors. For whereas the antitoxic antibodies merely combine with the toxins to form non-toxic components, the other antibodies not only fix the corresponding antigens, but bring about further changes.

Ehrlich very appropriately remarks that the mere fixation of *certain* food molecules would not suffice to render them available for purposes of nutrition, but that with molecules of large size, their destruction must precede assimilation. This could be effected, if the receptor in question had not only a haptophoric group "tuned"

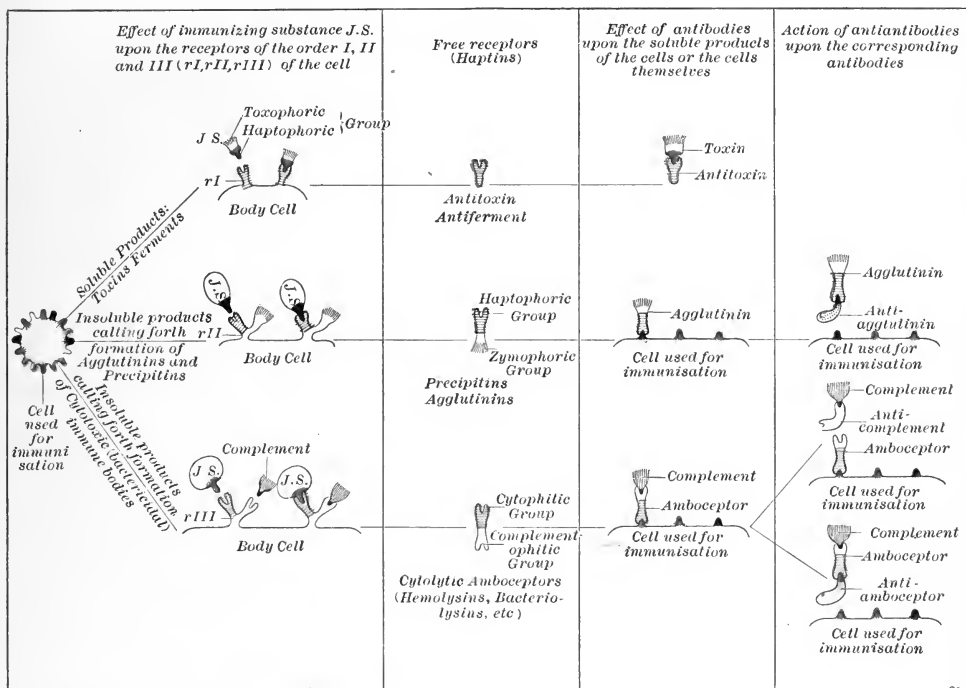
to the combining group of the food molecule, but in addition, either a second group of ferment character as part and parcel of the same receptor, or a second haptophoric group which might anchor ferment molecules, normally occurring free in the blood, when the other combining group is occupied by a food molecule of a certain structure.

Experimental investigation has shown that receptors of both types actually exist, and we may accordingly conclude that the antigens in question, like the toxins, do not represent true foodstuffs, but become anchored to the cells only because they happen to possess haptophoric groups which are identical in structure with those of the normal foodstuffs that the particular receptors are in the habit of binding. The consequence is that here, also, the cell will cast off the useless receptors and produce the same kind in unnecessarily large numbers, the excess being thrown off as in the case of the antitoxins. If, then, such free receptors meet with their respective antigens an interaction between the two will occur and this interaction will manifest itself by agglutination, precipitation, lysis, susceptibility on the part of a given cell to phagocytosis, etc., as the case may be. We should bear in mind, however, that the result, whatever it may be, cannot be viewed as being due to an interaction between the antibody and antigen as a whole, but as the antibody production is, no doubt, the outcome of the presence in the antigen of a definite molecular complex, the visible effect is merely the expression of an interaction between the antibody, and that particular group; agglutination, precipitation, and cellular lysis are thus purely secondary results.

In our illustration of the diversity of receptors which we conceive to exist in a given cell (Plate I), we have designated the essential differences in their general character by differences in color, and have assumed that receptors of one color can only combine with food molecules of the same color. The difference in the *structure* of the receptors is, however, best shown according to Ehrlich's original schema (see Plate II).

According to this diagram, then, we recognize three different kinds of receptors or *haptins*, as they are also called. Those of the *first order* possess only a single combining or haptophoric group, by which they unite with a corresponding group of the respective antigens. The antitoxins, antiferments, tropins, and anticomplements belong to this category.

PLATE II



Diagrammatic Representation of the Structure of the Different Antibodies and their Relation to the Corresponding Antigens. (Taken from Aschoff.)

The *receptors of the second order* likewise possess a haptophoric group for the corresponding antigen, and in addition a special *ergophoric* group, as it is called, by means of which the anchored antigen can be subjected to further change. To this group belong the agglutinins and the precipitins.

It will be noted that the receptors both of the first and the second order possess only a single combining group with which substances beyond the cell can unite. For this reason they are also spoken of as *uniceptors*. The *receptors of the third order*, on the other hand, possess two combining groups and are hence termed *amboceptors*. One of these is an ordinary haptophoric group which anchors the antigen to the cell, while the second combines with the complement of the serum and is hence spoken of as the *complementophilic* group. A special ergophoric group is not present, the changes which occur subsequent to the union between antigen and antibody being effected by the complement of the serum. To this order belong all the cytotoxins (or cytolytins), the immune opsonins, and the lipidophilic antibody of Wassermann.

From the above survey it is quite evident that Ehrlich's side-chain theory lends itself exceedingly well to experimental investigation, and it may not be out of place to consider in some detail how far the experimental facts support some of the more immediate conclusions to which the theory would lead.

We have seen that according to Ehrlich, the antibodies are not formed *de novo*, but that they are molecular groups which were once part and parcel of the cell upon which the corresponding antigen has acted. In that case it should be possible to neutralize the effect of the antigen by treating this with the cells from which the antibody is supposedly derived, and conversely we would expect that an admixture of other cells would not produce this result. Ransom was one of the first to investigate this point. After poisoning pigeons with tetanus toxin he found that extracts of all the organs were toxic, excepting those made from the central nervous system. This would suggest that the latter alone had been able to bind the toxin. Wassermann and Takaki then showed that this is actually the case, for on rubbing up the same toxin with the brain substance of guinea-pigs, and injecting the mixture into animals, no deleterious results were observed, while the liver, spleen, adrenal glands, muscles, etc., did not have this effect. These results are thus quite in accord with what we would expect on the basis of Ehrlich's theory.

By working with animals, on the other hand, which are either not at all, or but very slightly susceptible to the action of the toxin in question, such as the turtle, the frog, or the alligator, we would expect that the brain substance of these would have little if any neutralizing action upon the poison. With this supposition the facts are in perfect accord. Analogous results have been obtained by Kempner with the botulismus toxin, which like the tetanus toxin, possesses a specific affinity for the nervous system, and we have already seen that it is possible to remove hemolytic and bacteriolytic amboceptors from the respective sera by mixing these with their corresponding antigens.

The next question which Ehrlich's theory would suggest has reference to the experimental basis for the idea that the antibodies are actually formed by the cells which possess suitable receptors for the various antigens. Two possibilities present themselves in this connection. We can conceive, on the one hand, that the antibodies might be formed only by those cells whose functional nucleus can be deleteriously influenced by the antigen, while, on the other hand, the possibility exists that any cell may produce antibodies to a given antigen, providing only that it possesses a haptophoric group which is capable of uniting with the antigen. Investigations in this direction have led to the conclusion that a mere union between antigen and cell receptor is not always sufficient to call forth antibody liberation, but that a special "Bindungsreiz," or stimulus, must also be operative. Bruck thus found that on immunizing guinea-pigs with two separate solutions of tetanus toxin which were several years old, and of which one was still slightly toxic, while the other had lost its toxicity altogether, antitoxin production could only be elicited with the first. With the non-toxic specimen this was impossible, even though it could be shown that this still had the power of binding antitoxin, which means, of course, that its haptophoric group was still intact.

Bruck then argued as follows: If the nerve-cell receptors of the animal that has been treated with the non-toxic product, *i. e.*, with so-called *toxoid*, actually combine with this, then the subsequent injection of a dose of active toxin of such amount as would be just sufficient to cause death in a normal control, should now prove non-fatal. As a matter of fact this is exactly what occurs if the injection of the toxin follows that of the toxoid immediately. If, on the other

hand, an interval of twenty-four hours is allowed to elapse between the first and the second injection the latter will prove fatal, and it may further be shown that after such an interval a dose which would be subfatal for the control is now fatal for the toxoid animal.

The interpretation, of course, is that the toxoid which was first injected has not only combined with the nerve-cell receptors, but has actually called forth an increased production of new receptors of the same order, so that there is now present in the nervous system a larger number with which the toxin molecules can combine. The same experiment also shows that even though the toxoid has called forth such an increased production of receptors this was not followed by their liberation; for, if this had occurred, the toxin molecules would have met these in the circulation, union would have taken place there and the animal would have remained alive. We are thus forced to the conclusion that within certain limitations, antigens, of the toxin type at least, can call forth antibody liberation providing that the toxophoric group has not been entirely destroyed.

As regards the question whether antibody production can be effected only in those cells upon which the toxophoric group can exert a deleterious effect, or whether the same result can be reached with other cells, it is clear from the experiment cited above, to show that the toxins are actually bound by those cells which are susceptible to their toxic influence, that the antitoxins are also formed by these cells. This is further supported by an experiment of Roemer. This investigator rapidly immunized the right conjunctiva of a rabbit with abrin and then killed the animal. If now the right conjunctiva was triturated with a single fatal dose of abrin and the mixture was injected into an animal, no deleterious result followed. If, however, the same was done with the left conjunctiva the animal died. Roemer accordingly concludes that in the right conjunctiva locally formed antitoxin must have been present.

While the evidence is thus quite conclusive that cells which are susceptible to the toxic action of the toxin molecule may also produce antitoxins, there are other facts to show that this can also occur in non-sensitive cells. If, however, by any chance the sensitive cells are the only ones which possess the necessary combining group for the toxin, they will of necessity be the only ones from which antitoxin formation can proceed. We have seen already that in the case of the guinea-pig the nerve tissue is the only tissue which can

exercise a detoxifying action. In the case of the rabbit, on the other hand, such an effect can be produced not only by the cells of the nervous system, but also by the liver and the spleen. If, moreover, a guinea-pig is injected with tetanus toxin the fatal dose is the same, no matter whether the poison is injected intracerebrally, intravenously, or subcutaneously. In the rabbit the result is different. Intracerebral injection of tiny doses readily leads to fatal tetanus, while much larger amounts can be administered subcutaneously without causing a fatal result, particularly if the larger nerve trunks in the district in which the injection is made are previously cut. As abundant antitoxin formation then takes place notwithstanding the fact that the access of the toxin to the brain has been excluded so far as possible, the inference, of course, is perfectly warrantable that the antitoxin in question is largely produced by cells which are not susceptible to the toxic action of the poison.

The same point is also well illustrated in the case of the alligator. This animal is not at all susceptible to the action of tetanus poison. But notwithstanding this fact the toxin rapidly disappears from its blood after injection, and in its place large amounts of antitoxin appear. Were the toxin only physically stored away in the tissues, but not chemically bound, then we should not expect antitoxin formation, and the toxin should still be demonstrable in the tissues. This is what actually happens in scorpions. Metschnikoff injected such animals with a thousandfold quantity of the toxin as compared with that which is necessary to kill mice. The animals in this case were likewise not rendered ill and the toxin here also disappeared from the blood. But on testing for the presence of antitoxin none could be found, and even after months it could be shown that unchanged toxin was present in the liver. The interpretation of these findings upon the basis of Ehrlich's side-chain theory is very simple. Neither the alligator nor the scorpion are rendered ill by the toxin, because neither animal possesses cells that could be deleteriously influenced by the toxin. The alligator, however, produces antitoxin, because its cells are nevertheless able to enter into chemical union with the toxin, and it is for this reason that the toxin disappears from the circulation. The scorpion, on the other hand, has no cells at its disposal which could unite chemically with the toxin and it can hence produce no antitoxin. The poison here simply disappears because

it is physically absorbed, and it still remains active for this very reason and because it is not chemically bound.

A further consequence of Ehrlich's side-chain theory would be the inference that it should not be possible to call forth antibody production by immunizing with exactly neutralized antigen-antibody mixtures, for in such instances the haptophoric group of the antigen is supposedly in combination with the corresponding group of the antibody, and it should hence not be able to combine with any receptors in the body of the injected animal. Here also the facts are in accord with the demands of the theory. It is thus impossible to call forth any antitoxin formation with *accurately* neutralized toxin-antitoxin mixtures. It should be added, however, that in such experiments it is absolutely essential to have no free toxon present beside the toxin.

Toxons are poisonous bodies which may be present in the diphtheria bacillus cultures together with the toxins, but they differ from these in being less toxic. Like the toxins, however, they possess a haptophoric group which is capable of combining with the true antitoxin though the affinity of the toxon for the antitoxin is feebler than that of the toxin. Since the toxon effect is not acute, but only develops after a period of two weeks or longer, it is clear that *apparent* neutralization of a toxic bouillon, as tested by the non-development of acute symptoms, does not imply the absence of *toxons*; and as the latter contain the same haptophoric group as the toxins it is clear that a mixture of both, which is neutralized by antitoxin only so far as the toxins go, can still call forth antitoxin production. If, on the other hand, both toxins and toxons are neutralized, then, as I have pointed out, no antibody formation will take place.

Analogous experiments with cellular antigens and their corresponding antibodies have led to corresponding results, though these are not so striking, as in the case of the toxin-antitoxin mixtures. This, however, cannot be surprising, if it is borne in mind that conditions here are much more complex. We have pointed out before that an apparent paradox results when a constant quantity of bacteria is treated with increasing quantities of agglutinin (page 111), but we have shown that this finds a ready explanation on the basis of the existence of so-called partial agglutinins which are "tuned" to different agglutinable molecules in the bacteria, and that as a consequence the *apparent* binding power of bacteria for their agglutinins may be perfectly colossal. Under such circumstances one

could hardly expect to throw out of action *all* the agglutinable groups in a given quantity of bacteria by treating these with a corresponding agglutinin, even in very large amount. But notwithstanding this difficulty Neisser and Lubowski obtained some sera by immunizing with such mixtures, which contained no agglutinins at all. This, to be sure, was exceptional; but they could show, nevertheless, that in a *series* of experiments the subsequent agglutinative value averaged only 1 to 106 as compared with 1 to 1093 in the control animals, viz., in those which had been injected with non-agglutinated organisms.

On the basis of Ehrlich's theory the appearance of the so-called natural antibodies in the serum can now also be accounted for in a ready manner. Since the antibodies are not formed *de novo*, but merely represent normal molecular complexes of the body cells, it can hardly be surprising that once in a while, even in the course of normal events, some of these side chains will be cast off, although no bacteria or their toxins may have entered the body. That the antibodies, moreover, which result on immunization with foreign cells or cell products should be specific, is a necessary consequence, if we accept the view that antibody production presupposes the existence of a special affinity between the haptophoric groups of antigen and antibody. The remarkable point in this connection indeed is not so much the fact that the injection of a toxin should give rise to an antitoxin, or of bacteria to corresponding lysins or cytotoxins, but that so many varieties of antibodies should be possible for a given animal.

On the basis of Ehrlich's theory we are forced to conclude that the cells of the body collectively must contain at least as many different types of side chains *preformed* as the number of different antibodies that can be theoretically obtained from a given animal, and *vice versa*. This, however, does not seem altogether likely, if we bear in mind the innumerable varieties of antibodies that can actually be produced. I would only recall the possibility of obtaining specific precipitins to the albumins of almost all the different types of animals, then again the production of agglutinins not only to different species of bacteria, but even to different strains of a single species, etc. But it seems to me that even though we accept Ehrlich's theory in its essential points that we need not suppose the existence of such an enormous variety of receptors as occurring

performed. It would seem perfectly plausible that though *some* of the receptors, which we meet with as antibodies, may actually exist preformed, that others are developed only when certain antigens are brought in contact with certain cells, and in consequence of a special "Bildungsreiz." Experiments in this direction have, so far as my knowledge goes, not yet been made, but it should be possible to test the hypothesis just set forth. If my surmise were correct a still more plausible explanation of the specificity of the antibodies would thus be afforded.

Before concluding the present chapter one more point may yet be appropriately considered, viz., the question why those poisons which we can prepare in pure form in the chemical laboratory, and whose structural composition is known, such as the various alkaloids, glucosides, alcohols, etc., do not give rise to antibody formation. The fundamental reason for this differing behavior according to Ehrlich lies in the fact that the true antigens are chemically bound, and that chemical interaction between antigen and cell receptor takes place because the bodies in question are structurally closely allied to the true foodstuffs. The majority of poisons of the chemical laboratory, on the other hand, are not taken up by the cells in virtue of the existence of a special chemical affinity, but merely in consequence of physical influences.

This is well shown in the following experiment. After it had been discovered by Ehrlich and Overton that the injection of various anilin dyes leads to their storage in certain tissues of the body, and that this storage is due to the presence in these tissues of certain lipoids which act as solvents for the pigments in question, Hans Meyer and Overton could demonstrate that the strength of various narcotics is not dependent upon their chemical composition, but upon their coefficient of distribution which regulates their distribution between the blood plasma and the lipoids of the brain. This is well shown in the table on page 126, which is taken from Baum. The first column of figures represents the coefficient of distribution of the various narcotics, as calculated for water on the one hand, and fat on the other (calculated for olive oil), while the figures of the second column indicate the amount of the substances per liter, expressed in fractions of the corresponding normal solutions, which are just sufficient to produce narcosis in the test animal (usually frog larvæ); this is termed the *threshold of action*. By comparing

the two columns it will be noted that notwithstanding the wide variations in the chemical structure of the different narcotics, their effect is evidently dependent upon purely physical conditions, viz., the coefficient of distribution.

Narcotic.	Coefficient of distribution	Threshold of action expressed in fractions of the normal solutions.
	$\frac{\text{Concentration in fat}}{\text{Concentration in water}}$	
Trional	4.46	0.0018
Tetronal	4.04	0.0013
Butylchoralhydrate	1.59	0.002
Sulfonal	1.11	0.006
Bromalhydrate	0.66	0.002
Triazetin	1.30	0.01
Diacetin	0.23	0.015
Chloralhydrate	0.22	0.02
Ethylurethane	0.14	0.04
Monacetin	0.06	0.05
Methylurethane	0.04	0.4

In accord with this view regarding the action of the majority of the chemical poisons upon the cells of the body, is also the fact, that these substances can again be extracted from the cells by the use of appropriate solvents, which, of course, would not be possible if chemical union had taken place.

We may thus sum up by saying that only those substances can possess antigenic properties which are capable of entering into chemical union with the cells, but that in addition a special "Bindungsreiz" must be exercised upon the cell, which is peculiar to the antigens.

CHAPTER IX

THE DIFFERENT TYPES OF IMMUNITY

WE have seen in the foregoing chapter how satisfactorily Ehrlich's theory accounts for the formation and specific action of the antibodies, and thus for the origin and mode of action of some of the most important defensive factors of the animal body. Upon this basis we may now also take up for consideration some of the more general aspects of the problem of immunity.

When we speak of *immunity* in the biological sense, we understand thereby the existence of a certain resistance toward deleterious influences. This may be directed against a large number of factors, such as the action of various drugs and chemicals, the harmful effect of atmospheric conditions, attack by other animals, various degenerative influences arising from within the body, infections with vegetable or animal parasites and the absorption of their products of metabolism or degeneration, etc. From a medical standpoint, of course, these latter influences interest us particularly, and in the following pages we shall devote our attention to the subject of immunity from this standpoint more especially.

Natural Immunity.—The very fact that animal life is possible at all, surrounded as we are by organisms which under certain conditions can invade the body and cause its destruction, shows in itself that every individual must possess a certain degree of natural immunity. Staphylococci are thus found not only on the outer surface, but even in the deeper layers of the skin without giving rise to any disturbance; pathogenic pneumococci, streptococci, and even diphtheria bacilli may be present in the fauces without producing disease; the intestinal canal is inhabited by untold millions of bacteria, some of them of pathogenic character, which apparently produce no deleterious effects, and so on. *Apparently* the individual who normally harbors all these various organisms is immune to the corresponding infections.

The picture, however, changes very materially, if in some manner

a break in the continuity of the epithelial lining of the outer or inner surfaces of the body occurs. A surface bruise may be followed by the formation of an abscess, an injury to the nose may lead to meningitis, the irritation of the gall-bladder by a calculus may be followed by cholecystitis. The gardener or the stable man may have his hands soiled by material containing tetanus bacilli without any harm, while the infliction of a trifling wound may lead to fatal lockjaw. Evidently the immunity to certain diseases which one would infer from the presence of the corresponding pathogenic organisms on the surface of the body in the absence of symptoms of disease is only apparent.

All that we can infer from such observations is that the surface epithelium shows a certain degree of resistance to infection, *i. e.*, that in a certain sense at least it is immune. Numerous observations go to show, as a matter of fact, that local conditions play an important role in determining the degree of resistance to infection. It has thus been demonstrated that certain organisms can only infect when they are introduced in a certain manner, while others can do so from practically any point. The pathogenic cocci and plague bacillus are examples of the latter kind, while the dysentery bacillus, the cholera vibrio, and certain meat-poisoning bacilli require a special portal of entry. We may then conclude that the body possesses virtually no tissue immunity toward the first and a fairly high grade of immunity toward the second order. Quite in accordance with these observations is the fact that in certain animals tetanus can be produced only by intracerebral injection of the corresponding toxin, while in others the disease develops, no matter what the character of the tissue may be in which the injection is made.

The same point is also well illustrated by the remarkable predilection which certain organisms have for certain organs, when once they have passed the outer epithelial barriers. If young rabbits are thus injected intravenously with cholera vibrios they die after a few days, and post mortem the organisms are found in large numbers in the intestinal mucosa, while the blood and remaining organs are sterile (providing, of course, that the number injected has not been unduly large). Evidently the intestinal mucous membrane offers little or no resistance to the cholera vibrio, while the other tissues show a considerable degree of immunity. Well known, also,

is the marked affinity which exists between the meningococcus and the meninges, of the pneumococcus for pulmonary tissue, of streptococci for serous membranes, of the typhoid bacillus for lymphoid structures, etc.; while the other tissues show a more or less well-defined immunity. Evidently the degree of resistance or immunity which the animal offers to infection depends both upon the nature of the organism and the route by which it is introduced.

If infection, by what we may term a natural route, is excluded, then there will be an apparent immunity, at least, to the organism in question. For practical purposes this type of immunity may, indeed, be regarded as absolute. But that it is not so of necessity can in some instances be demonstrated by introducing the organism through channels by which natural infection would not be likely to occur. In the human being, typhoid infection will thus almost always develop by way of the intestinal canal. In most of our laboratory animals, infection by this channel is impossible, and we might accordingly regard them as immune. That this is only apparently the case, however, can be readily shown by injecting the organisms intraperitoneally, when a fatal infection can be produced at will. The fact remains, nevertheless, that the various animals in their natural state do not contract typhoid fever, although they must be exposed to infection on many occasions. They may hence be regarded as *practically* immune. Many instances of immunity no doubt are dependent upon such causes, viz., upon the existence of immunity of those tissues by which natural infection would ordinarily occur.

Class Immunity.—Generally speaking, the natural susceptibility to infection by microorganisms differs with the different classes of animals, with different genera, with different species, and even with different varieties and individuals. We accordingly recognize a natural class immunity, a natural generic and species immunity, natural race immunity, and individual immunity. Class immunity is especially interesting because it presents examples of *absolute immunity*, under natural conditions at least, which is, after all, exceedingly rare. The immunity of cold-blooded animals toward the majority of those organisms which are pathogenic for warm-blooded animals belongs to this order. But even here the immunity is sometimes only relative and apparent. The frog is thus *naturally* insusceptible to anthrax, and the injection of large numbers of such

organisms will under ordinary conditions produce no deleterious results. If, however, the animals are kept at a temperature at which anthrax bacilli can readily grow, infection promptly takes place. Conversely, Pasteur found that it is possible to infect chickens with anthrax, by refrigeration, whereas normally the animal is immune.

Toward the leprosy bacillus there is apparently an absolute immunity not only on the part of the cold-blooded animals, but also of the vertebrates, with the exception of man and possibly of certain monkeys.

Generic Immunity.—As examples of generic immunity we may mention the resistance of man to the common organisms which are pathogenic for the lower vertebrates, and *vice versa*.

Species Immunity.—Species immunity is illustrated by the resistance of dogs, pigs, and rats to the anthrax bacillus, while cattle, sheep, and most of the common laboratory animals are quite susceptible to infection with this organism. Cattle plague (rinderpest), swine plague (Schweinerotlauf), sympathetic anthrax (Rauschbrand), chicken cholera, etc., do not affect man under normal conditions, while animals are naturally immune to infection with the cholera vibrio, the meningococcus, the typhoid bacillus, the gonococcus, the treponema pallidum, as well as to such diseases as scarlatina, measles, yellow fever, poliomyelitis, etc.

Racial Immunity.—Racial immunity is exemplified by the relatively high degree of resistance of Algerian sheep to anthrax, to which our own domestic sheep are very prone. Black rats are more resistant to anthrax than gray rats and gray rats more so than white rats. The same point is also well shown in the remarkable difference in the susceptibility of different races to such diseases as measles, smallpox, tuberculosis, etc.

Individual Variations in Susceptibility.—The occurrence of individual variations in the susceptibility to various diseases, further, is so well known as hardly to require special mention. During epidemics of cholera, smallpox, diphtheria, yellow fever, typhoid fever, influenza, etc., this is particularly noticeable. There are then always some persons who escape infection even though they have been freely exposed, and among those which develop the diseases in question there are some in whom the malady runs a mild course, while others are fatally stricken; in some we see a remarkable

tendency to complications, while others recover without any untoward incident, and so on. We must accordingly conclude that some individuals are naturally immune to certain infections and that even among those who are attacked there must be marked quantitative variations in resistance.

Acquired Immunity.—The different types of immunity which have been briefly considered above have one point at least in common—namely, the fact that they exist under natural conditions, and we hence speak of immunity of this order as *natural immunity*. Without entering into a discussion of the possible etiological factors which may have been operative in the production of this type of immunity, we may emphasize that it apparently does not depend upon a process of active immunization, viz., upon the introduction either of the pathogenic organism or its product. This is in marked contradistinction to another type of immunity which is directly dependent upon these very factors and which we accordingly speak of as *acquired immunity*.

It has long been recognized that individuals who have once passed through certain diseases, such as smallpox, chicken-pox, scarlatina, measles, mumps, whooping cough, typhoid fever, typhus fever, yellow fever, and Asiatic cholera, are subsequently immune either absolutely, or to a very considerable extent. The recognition of this fact has been of the greatest importance, for it forms the basis of our modern attempts to create an artificial immunity to different diseases, or if not an actual immunity, then at least increased resistance by the purposeful introduction of the corresponding infecting agent in such form as not to expose the individual to the dangers of natural infection. We may thus distinguish between an *artificially acquired immunity* and what we may appropriately term *accidentally acquired immunity*.

The discovery of the possibility of producing immunity artificially we owe to Jenner, who first showed that by “vaccinating” individuals with smallpox virus which had been attenuated by passage through cattle, protection against the dreaded malady could be secured (1798). Although the causative agent of smallpox was unknown, Pasteur subsequently recognized that the principle of vaccination lies in the production of the disease in an attenuated form. The thought hence suggested itself to him that the same principle might be adapted to the prevention of bacterial diseases also, and by

experimentation in this direction he laid the foundation of our modern vaccine therapy, which finds its most important expression, so far as human pathology is concerned, in the curative (sc., preventive) treatment of rabies, and in the prophylactic vaccination against typhoid fever. In the laboratory it has further led to the recognition of the fact that even though immunity cannot be produced against all pathogenic organisms by vaccination, it is at least possible to bring about a marked increase in resistance, and by applying this principle to other infectious diseases, to which man is subject, a radical advance in the rational treatment of these maladies has been achieved (see section on Vaccine Therapy).

Antitoxic Immunity.—In a previous chapter we have seen that some pathogenic organisms injure the host into which they have been introduced through the products of their metabolism or degeneration, insofar as these are of toxic character, while their infectiousness may be of a very low order. Others produce a harmful effect directly in consequence of their high grade of infectiousness, even though they do not give rise to toxic products, while in still other cases we see both factors variously combined. Evidently, then, the existence of a natural immunity, or of immunity brought about as a consequence of infection, may manifest itself either as a resistance of variable degree against the development of microorganisms in the body of the infected animal, or as a resistance against bacterial toxins, endotoxins, aggressins, etc., or it may be directed against both. It is, hence, appropriate to speak of *antitoxic immunity* on the one hand, and *antibacterial immunity* on the other.

As an example of *natural antitoxic immunity* we may mention the natural resistance which the alligator offers to the action of tetanus toxin, while the steadily increasing resistance to diphtheria toxin manifested by a horse undergoing corresponding immunization may serve as an illustration of *acquired antitoxic immunity*. The natural resistance of rats and dogs to anthrax, on the other hand, is of antibacterial character, as is also the immunity or increased resistance, at any rate, which results on vaccination with the same organism in otherwise susceptible animals, such as sheep, guinea-pigs, and mice.

If an individual becomes immune to a given organism or its toxic products as a result of infection or vaccination, in consequence of his own efforts, as it were, we speak of *active immunity*, while immu-

nity which results from the transference of protecting substances from an immune animal to a non-immune individual is designated as *passive immunity*. The difference between the two is well illustrated, if we compare the recovery of a diphtheria patient without treatment, with the recovery of one which follows as a consequence of the administration of antitoxin.

In the first instances the patient recovers because he succeeds in forming enough antitoxin in his own body to neutralize the toxin produced by the invading organism, pending the destruction of the bacteria by other means, while in the second the patient is protected against the deleterious effects of the toxin through the introduction of the corresponding antitoxin from without. The possibility of passive immunization is, of course, of the utmost importance as successful serum therapy *during the actual progress of a disease* is dependent upon this principle, while active immunization in the nature of things forms the basis of prophylactic vaccination.

Mechanism of Different Types of Immunity.—If now we turn to a consideration of the mechanism which underlies the different types of immunity, as just outlined, various possibilities suggest themselves.

Aside from those factors which render the actual invasion difficult if not impossible, such as the character of the epithelial covering and the nature of the secretions which are poured out upon the epithelial surfaces, the chemical and physical characteristics of the medium in which the organism finds itself after invasion has taken place are of necessity determinative for the question whether infection will or will not occur. These factors, of course, may be entirely independent of any direct bactericidal action of the body cells and juices *per se*, and have to do simply with the character of the environment, viewed as a culture medium for the organism in question.

We know that certain organisms can develop successfully *outside of the body* only, if the temperature, the reaction of the culture medium, and its chemical composition are of a definite character. The remarkable fastidiousness in this respect of such organisms as the gonococcus and the influenza bacillus is well known. It is accordingly quite conceivable that infection with certain organisms cannot occur because of the unfavorable character of some factors of this order *within the body*. The effect of temperature in this respect is thus well shown in the case of cold-blooded animals, like the frog, which is naturally immune to infection with the anthrax bacillus,

but which loses its resistance when kept at a temperature at which the organism will normally develop outside of the body. Conversely it has been noted that frogs which under natural conditions, *i. e.*, at low temperature, readily fall a prey to infection with the bacillus *ranicida*, are immune to the same organism if kept at a temperature of 25° C. Of the same order, no doubt, is the immunity of chickens to anthrax, which disappears when the animals are kept immersed in water of 25° C., their normally high temperature being reduced in this manner. In cases such as these the *modus operandi* of the temperature changes upon immunity or infection *seems* relatively simple, while in others it is certainly of a more complex order.

It is thus a well-known fact that man and other animals after exposure to cold are more prone to infection with a number of different organisms, which find their optimum condition for growth at the normal temperature of the body. The underlying causes of the change in resistance in such cases are apparently different, but what the mechanism is we do not know. We can readily imagine, however, that functional disturbances may be set up in the macroörganism by the cold which in some manner operates to the advantage of the microörganism. It is not excluded, of course, that in the instances of immunity mentioned above, something similar may not also be operative, but the simple explanation that has been offered cannot be overlooked.

Athreptic Immunity.—In other cases the resistance to infection may be referable to the existence of unfavorable conditions of nutrition. A number of observations have taught us that certain organisms require certain specific foodstuffs for their development, in addition to others which are necessary to all forms of life of that order, and unless these are present, successful growth cannot take place and immunity would accordingly result. Immunity of this type is spoken of as athreptic immunity.

Ehrlich first suggested this term to denote the peculiar behavior of mouse cancer when transplanted into rats. At first active growth takes place, so that at the end of eight to ten days the size of the tumor does not differ from control tumors in mice. After that, however, further growth ceases and resorption takes place. If, now, *i. e.*, at a time when active growth no longer occurs in rat *A* a transplant be made to another rat *B* the graft does not develop. But if a mouse be inoculated instead, active growth takes place, and

if from this a transplant is made to rat *B* a tumor develops as in rat *A*. To explain this peculiar behavior Ehrlich suggested that some specific substance which we may call *X*, and which is supposedly found only in the body of the mouse, and which is essential to the growth of the mouse cancer, is transferred to rat *A* when the first transplantation is made. As long as a supply of this substance is available the cancer cell can multiply and make use of the usual foodstuffs of the organism of the rat. As soon as this is exhausted, however, further development is not possible, and if at this time rat *B* is inoculated no growth occurs because the specific growth stuff *X* is absent. If a transplant be made back to a mouse, however, *X* is again supplied and a transfer to rat *B* will then again lead to successful growth until *X* is again used up. The immunity of the rat to the mouse cancer is thus evidently dependent upon an *athrepsia*, *i. e.*, an absence of a specific substance which is essential to the growth of the mouse tumor cells. This concept of a certain form of *tumor immunity* is theoretically, at least, applicable to certain types of antibacterial immunity also, even though the experimental basis for such an assumption has not yet been supplied.

We know, of course, that certain organisms can be grown outside of the body only, if certain special substances are supplied and that in their absence growth ceases. A familiar example is furnished by the influenza bacillus. If this is transplanted from hemorrhagic sputum to ordinary culture media a certain amount of growth is at first obtained, but unless hemoglobin is artificially supplied to the subcultures the organism soon dies out. We may accordingly imagine that certain animals are immune to infection with certain organisms because the macroörganism does not supply all those substances which are essential to the growth of the microörganism, but, as just stated, we do not as yet know what those substances are, and we do not know against what organisms an athreptic immunity exists. We merely recognize the possibility and must reckon with it in our discussion of the subject.

Antiaggressin Immunity.—Another factor which must be considered in connection with the question regarding the mechanism, which is operative in the production of antibacterial immunity, is the possibility that the organism, which has found its way into the body, may be devoid of all aggressivity, and that it hence falls an easy prey to the normal defensive mechanism of the macroörganism.

Here also our knowledge is as yet very meager, but it would seem that this possibility actually exists. In the case of the anthrax bacillus, for example, it has been ascertained that by suitable methods the organism can be deprived of its power to form capsules and that such strains are then no longer capable of producing infection. We have seen before that this organism owes its infectiousness to a large extent to its ability to surround itself with a capsule, and that when once encapsulated it is no longer open to successful attack by the phagocytes. It is thus easy to see why an animal should prove immune to infection when an organism is introduced which depends for its existence in its new environment upon aggressive factors of this order and is incapable of developing them.

While immunity of this type would depend upon lack of aggressivity in the more general sense on the part of an organism, there is evidence to show that immunity may also be due to the same factor in the more restricted sense of Bail, viz., upon an inability of the organism to overcome the normal defensive factors by the secretion or liberation of soluble aggressins. This is well illustrated in the case of the pigeon, which is markedly immune to anthrax even though its serum *per se* is not bactericidal for this organism. Upon the addition of leukocytes, however, it becomes so, and against this combination an amount of anthrax aggressin is powerless which would suffice to overcome the bactericidal power of a corresponding serum-leukocyte mixture taken from a guinea-pig which itself is markedly antiaggressive in its action. Of the manner in which this effect is produced, however, we know nothing.

We denote this type of immunity as *antiaggressin immunity* merely to express the fact that it depends upon factors which are not of a bactericidal nature, but which prevent the development of those aggressive functions upon which certain organisms depend for their existence, after invasion of the body has taken place. The animal is immune not because it has stronger bactericidal forces either in its serum or its cells, not because it can prevent the animalization of the invading organisms, not because of any antitoxic mechanism, but because the organisms for some reason find themselves incapable of exercising their special aggressive forces. But of the reasons why this should be so in one animal and not in another, we know nothing.

While the existence of an antiaggressin immunity in the special sense of Bail, as just outlined, has thus far been established only

in a single one of the naturally immune animals, it is not unlikely that the same mechanism may be operative in the production of natural immunity in others, and especially in connection with those organisms which, like the anthrax bacillus, are characterized by a high degree of infectiousness and a low grade of toxicity. In the case of some of these, it probably also plays a role in the development of an acquired immunity.

Antibacterial Immunity.—In the majority of infections with the semiparasites, on the other hand, the acquired immunity is not antiaggressive, but bactericidal in character, and since bactericidal influences may be exercised either by the serum alone or the leukocytes alone, or by both in combination, the resultant immunity may, theoretically at least, be due to an exaggerated functional activity of either one or both of these factors. It is not my purpose at this place to enter into a discussion of the question which one of the two is really the *primum movens* in the production or existence of *antibacterial immunity*. I would merely recall that for many years, immunity students were divided into two opposing factions, viz., the *humoral school*, led by Pfeiffer, and the older *phagocytic school*, represented by Metschnikoff, whose respective standpoints seemed for a long time irreconcilable the one with the other. At the present time the original sharp lines between the two schools have fallen, and we recognize that there is an intimate interrelationship between the cellular and the humoral defences, that the two supplement one another and that neither alone should be viewed as sufficient to protect an animal against infection and its consequences. But while recognizing the importance of both, we must also admit that neither the one nor the other seems to be solely responsible for the development of an acquired immunity.

There can be no doubt that as a result of infection or vaccination, corresponding bacteriolytic amboceptors are formed in large quantity and that the serum of such animals in the test-tube is capable of destroying the corresponding organisms in large numbers, and that the same can occur in the living animal, but we must also recognize the fact that this flood of bacteriolytins does not remain while the increased resistance that has been established may last for years. That the phagocytic influences in such cases do not play a more important part than the bacteriolytins, can readily be shown by studying the opsonic curve, which never remains above the normal

for any length of time after the infection has come to an end, if indeed it has been increased at any time during its course, or thereafter. Evidently, then, still other influences must here be operative, but what these influences are is still a matter of speculation. If we bear in mind that a cell which has once been stimulated to active antibody formation, probably responds to subsequent stimuli of the same order with greater rapidity, and that those receptors no doubt are regenerated in greatest number which have the greatest affinity for the particular antigen to which they are "tuned," we can imagine that the introduction of the corresponding organisms at any period following the original infection or vaccination will be successfully overcome in consequence of this specially active response.

This, however, is as yet a mere supposition, and the question still remains unanswered why infection with certain organisms leads to immunity and not with others. A discussion of the many possibilities which present themselves in connection with this problem would serve no useful purpose at this place. Much work still remains to be done, but the main avenues along which profitable research should be conducted are already clearly indicated.

Mechanism of Antitoxic Immunity.—While our knowledge of the mechanism underlying the development of antibacterial immunity is thus still very fragmentary, and really permits a clearer insight into the manner in which infection can take place than into the reasons why immunity may or may not develop, we have a much better understanding of the *modus operandi* which forms the basis of the antitoxic type of immunity. The organisms which are characterized by a high degree of toxicity, such as the tetanus and the diphtheria bacillus, as we have repeatedly pointed out, possess a very low grade of infectiousness, so that they readily succumb to the normal bactericidal agencies of the body. Their toxicity, however, is of such a high order that they are nevertheless formidable pathogenic agents. It is accordingly surprising to find that some animals are absolutely immune to the action of these toxins, and, as a matter of principle, it is important to learn to what agencies this remarkable natural immunity is due. In this connection Ehrlich's side-chain theory regarding the origin and formation of antibodies has been very helpful in arriving at a fairly definite understanding.

Different possibilities, of course, suggest themselves. Since a toxic effect presupposes the existence on the part of some of the body cells of special molecular groups with which the toxins can combine, it stands to reason that a natural absence of such groups must lead to natural immunity so far as that special toxin is concerned. But if this be the case then the formation of a corresponding antitoxin should not be possible. By this criterion, then, we can test any cases that might suggest themselves as belonging to this order. Metschnikoff has pointed out that certain reptiles, and notably the turtle, are naturally absolutely immune to tetanus toxin; no matter whether the animals be kept at the ordinary temperature of the aquarium, or at 37° C., following an injection of the toxin, their blood remains highly toxic for mice, even for several months. Coincidentally he found that there was not the slightest formation of antitoxin. This example then illustrates especially well the actual existence of a theoretically possible form of *immunity due to absence of suitable receptors*.

A second possibility suggests itself if we bear in mind that not all cells which may possess suitable combining groups for a toxin molecule are necessarily deleteriously affected by such a union. In such an event we should expect absence of toxic effect associated with the production of antitoxin, for we have seen that the latter can take place perfectly well even though the specific action of the toxophoric group is eliminated. That this may actually occur in nature is well shown in the case of the American alligator, which is as resistant to the action of the tetanus toxin as is the turtle, but which, unlike the latter, furnishes an abundant amount of corresponding antitoxin. That the toxin in this case is actually bound by the cells is also shown by the fact that, contrary to what we have noted in the turtle, it rapidly disappears from the circulation. In such a case the immunity is evidently not due to absence of suitable receptors, but to an *insusceptibility on the part of the binding cells*.

Still another possibility would exist, if both susceptible and insusceptible cells were present in the body, but if the latter possessed a greater affinity for the toxin than the former. In such a case we should theoretically expect active antitoxin formation, immunity to small doses of the toxin, but absence of immunity to a larger dose, the result, moreover, varying with the point at which the

toxin is introduced. If this should occur in a territory which contains large numbers of insusceptible cells (though provided with suitable haptophoric groups) no deleterious results would be expected, while in the opposite case the consequences would of necessity be disastrous. A great deal, moreover, other things being equal, would depend upon the size of the dose, for if this should exceed the demands of the insusceptible cells a toxic effect would naturally be the outcome. In such instances, then, the immunity would only be relative. This is exactly what we see in the case of the rabbit, which is relatively insusceptible to tetanus toxin, when this is administered hypodermically, but highly sensitive if the poison is injected directly into the brain.

It will be noted that these three different types of immunity are thus essentially dependent upon the character of the cells, *i. e.*, that they are *histogenetic* in character, and that the examples which have served as illustrations at the same time represent types of natural immunity. But we have also seen that immunity (*sc.*, increased resistance) must result if for any reason antitoxin molecules enter the circulation in sufficient number to neutralize any toxin that may be present. Immunity of this order is thus *humoral* in character and usually, if not always, acquired. This may result as a consequence of infection or immunization, and then represents a type of *active immunity*, or it is acquired in a passive manner, the organism of the individual taking no part in its production (*passive immunity*). An example of the first type is furnished by the antitoxin horses, in which a high degree of immunity is produced by systematic immunization, while the production of passive immunity is illustrated in the prophylactic treatment of diphtheria or tetanus with the corresponding antitoxic sera. To the latter order also belongs the immunity which is conveyed by actively immunized animals to their offspring, either during intra-uterine life or post partum through the milk.

While the underlying principle of these types of immunity is thus quite well understood, still another form of acquired immunity is, theoretically at least, possible. We have seen that under *natural* conditions a form of antitoxin immunity exists, which is referable to absence of suitable haptophoric groups on the part of the body cells. Theoretically it is conceivable that such a form of immunity might also be acquired, if in any way atrophy of the corresponding

receptors were to occur either in all cells, or only in those cells which are susceptible to toxin influence. As a matter of fact, there is experimental evidence to show that this may occur. Thus, while the red blood corpuscles of the normal rabbit are readily destroyed by the peculiar toxin which is found in the serum of eels, the corpuscles of correspondingly immunized animals, even though washed free from any antitoxin that may be present in the serum, are absolutely resistant. Evidently they have lost the receptors which in the non-immune animal made the action of the toxin possible. It has similarly been observed that animals which have been highly immunized against diphtheria toxin may finally cease the production of antitoxin altogether, and simultaneously lose their susceptibility to the toxin in question altogether, phenomena which are most readily explained upon the basis of acquired atrophy of the corresponding receptors.

While these examples plainly illustrate the undoubted occurrence of an acquired *antitoxic immunity*, due to *receptoric atrophy*, there are further observations which show that this principle also plays an important role in the development of other types of immunity. Thus far we have only considered the reaction of the macroörganism to the introduction of the microörganism, and the question very naturally suggests itself, Is it not possible that the microörganism may become resistant to the deleterious influences which it meets within its host? We have seen that it may protect itself by the development of capsules and the liberation of aggressins. Within recent years, observations have come to light, however, which make it very probable that the principle of receptoric atrophy may play an important role here also. Much of this work and its brilliant interpretation we likewise owe to the genius of Ehrlich. He has shown that on treating rats which have been infected with trypanosomes (*SI*) with an amount of arsenophenyl glycin, arsanil, or arsacetin, not quite sufficient to kill all the organisms, trypanocidal antibodies are produced, which Ehrlich conceives to be the outcome of the antigenic effect of the ordinary nutriceptors¹ of the parasite, upon those cells of the macroörganisms which are provided with corresponding haptophoric groups. Those trypanosomes which have not been killed by the arsenic now find themselves in the presence of

¹ Nutriceptors are here understood to be those receptors which serve the nutrition of the organism (sc., the cell).

these antibodies (*A I*), and insofar as they are not destroyed, they respond to the occupation of their original nutriceptors (*N I*) by these antibodies with the production of a new type of nutriceptors (*N II*), which we may imagine to possess a greater affinity for the available foodstuffs than for the antibodies that are simultaneously present. A new strain of trypanosomes thus develops in which this peculiarity is handed down from each individual parasite to its descendants. If this strain (*S II*) is now tested against a serum containing antibodies of the type *A I*, it will be found immune, and as Ehrlich has pointed out, this type of immunity can be explained only in the manner just outlined, viz., on the basis of receptoric atrophy.

The importance of this principle in the interpretation of various phases of human and animal pathology is, of course, evident. It readily explains, for example, why the syphilitic individual is refractory to reinoculation, while he is liable to relapses starting from his original infection. We may imagine that in such a person, different strains of spirochetes develop as a result of adaptation to those antibodies which are formed in consequence of the death and absorption of the first and subsequently developing strains, and that the latest strain, in point of time of development, will always be capable of causing a relapse, as no suitable antibodies to it have as yet developed and because it is immune to those that have been formed before. The number of strains which can theoretically be produced in the course of an infection will, no doubt, vary with different organisms, as well as with the nature of the host. A great deal of additional work will have to be done, however, before we can speak with any degree of definiteness on this subject. Ehrlich has shown that in the case of the spirillum of relapsing fever only three or four strains are possible. If, then, the patient or animal has had two or three relapses the body will contain all the different "strains" of spirilloccidal antibodies that are possible, no new strain can accordingly develop, and spontaneous recovery will occur. The greater the number of strains which can develop the greater will naturally be the obstacles to spontaneous recovery. This holds good especially for such diseases as syphilis and trypanosomiasis (sleeping sickness), and possibly also for malaria.

From these brief considerations it will be seen that the subject of immunity referable to receptoric atrophy is a most important one, and that we may reasonably expect much valuable information

from a continued and more detailed investigation of the subject. Since the same principle, moreover, seems to apply not only to immunity to infection, but also to the question of resistance to various chemical agents on the part of various low forms of animal and vegetable life, it is clear that the subject must also be of great interest from the standpoint of therapeutics, and furnishes a logical basis for the now generally recognized fact, that in the medicinal treatment of certain infections, like syphilis, our aim should be a *therapia magna sterilisans*, rather than the continued administration of small doses of certain drugs (see section on Chemotherapy).

In fine we may say that much has already been learned of the manner in which immunity may develop, but much more still remains to be known. The avenues along which further investigations may be profitably pursued are already well defined and we may confidently expect much valuable new information in the near future.

CHAPTER X

ANAPHYLAXIS

It has long been recognized that while certain infections, such as smallpox, scarlatina, measles, whooping cough, typhoid fever, cholera, typhus fever, etc., lead to immunity, others not only bring about no increased, but actually a decreased resistance to subsequent infection with the same organism. This is notably true of pneumonia, erysipelas, influenza, diphtheria, bacillary dysentery, certain staphylococcus infections, such as tonsillitis, acne, etc. In the past we have been totally unable to explain these peculiar differences, and even now our knowledge of the mechanism underlying the production of *hypersusceptibility* to certain deleterious influences is very meager. Within recent years, however, such a wealth of experimental facts has been accumulated which have a direct bearing upon the problem under consideration, that the day no longer seems far distant when we shall be able to offer an adequate explanation for these peculiar differences. Some of these observations and the resulting deductions will be considered in the present chapter.

In the foregoing pages we have explained the manner in which immunity to toxins may be artificially brought about, and have shown that this depends essentially upon the liberation of corresponding receptors on the part of some of the body cells. If these are produced and thrown off in sufficiently large number, they furnish a protection for the body against the toxins in question which may be of a very high order. In the commercial preparation of antitoxins it is, of course, desirable to obtain sera that shall be as potent as possible, and it is hence customary to force the process of immunization in the experimental animals to the highest limit. The question now arises what happens if this be exceeded. Two events may then occur. On the one hand the animal may cease to produce antitoxin altogether, but simultaneously loses all susceptibility to the corresponding toxin, and is thus absolutely immune.

This result, as we have already shown, is explained on the basis of an *acquired receptoric atrophy*, and we can readily conceive that this

should occur, if the specific receptors which the cell forms under the stimulus of the toxin are continuously cast off, and thus no longer serve a useful purpose so far as the nutrition of the cell is concerned. On the other hand, the opposite may occur. The animal, while actively forming antitoxin, loses its increased resistance to the corresponding toxin, and succumbs to a much smaller dose of the latter than the original minimal fatal dose. It is thus no longer immune, but actually hypersensitive. As this phenomenon is only observed in *actively* and never in *passively* immunized animals, the conclusion suggests itself that its basis must be histogenetic and not humoral in character. Since antitoxin is present in the blood of the animals in large amount we must suppose that this actually anchors the toxin, but as the animal dies with typical toxin symptoms we must also conclude that the toxin-antitoxin combination is again severed and that the toxin after all reaches the corresponding receptors of the susceptible cells. This, of course, would presuppose the existence of a higher affinity for the toxin on the part of the sessile than of the circulating receptors. That there is actually a basis for such an assumption has been shown by Müller, who could demonstrate that at any one time the blood serum of an animal undergoing immunization contains antibodies of varying degrees of affinity for the corresponding antigen, and that those possessing the highest affinity are the latest formed.

The hypersusceptibility of the highly immunized animal would thus find a ready explanation, which would also seem to apply in the case of the so-called *paradox of Kretz*. This investigator found that while the injection of an accurately neutralized toxin-antitoxin mixture produces no deleterious results whatever in the *normal* animal, in one which has been previously actively immunized with toxin the reverse occurs. Here also we may suppose that as the result of the immunization, highly active receptors are present in the susceptible cells, and that these are capable of displacing the antitoxin which has been added *in vitro* and of anchoring the liberated toxin which then acts upon the cell before this has cast off the corresponding receptors.

If coincidently in either one of the two instances just considered receptoric atrophy should develop in the non-susceptible cells it is clear that the susceptible cells would become even more liable to attack by the toxin.

Probably belonging to the same order of cellular hypersusceptibility is also the *increased susceptibility of the tubercular organism* to the introduction of tuberculin in doses which in the normal individual produce no reaction whatever. We may here imagine that in tubercular foci, sessile receptors are present in large numbers which possess a greater affinity for the tubercular antigen (tuberculin) than do the receptors of any normal cells, and that these receptors eagerly take up the corresponding antigen, when this is introduced from without. The specific reaction which then takes place we can conceive to be due to an interaction between antigen (tuberculin) and antibody (receptor), with the consequent production of toxic products and their action upon the cells in question. This view is supported by the discovery on the part of Wassermann and Bruck that tubercular organs actually contain specific substances which will combine with tubercular antigen, as can be demonstrated with the complement fixation method (which see).

Richet's Early Investigations.—A marked impetus to the study of hypersusceptibility was then given by certain observations of Richet (1902). This investigator found that the intravenous injection into dogs of extracts made from the tentacles of certain actiniae produced marked toxic symptoms (excitement, bloody diarrhea, and subnormal temperature), which appear after a certain interval, then increase in severity during the first two days, and lead to a fatal issue only at the expiration of the third day. Post mortem he found marked congestion of the viscera (stomach, intestines, liver, and kidneys), and he accordingly termed the toxic principle in question *actinocongestin*. He further ascertained that very curiously the *immediate* repetition of a fatal dose of the poison never produced *sudden* death, but that the end was invariably delayed until the expiration of the third day. If now an animal is injected with a non-fatal dose of the poison, and after recovery from its effects is reinjected with an amount which in an animal that had previously not been injected would produce no deleterious effects whatever (such as one-twentieth of the original quantity), most serious symptoms develop at once and the animal dies within twelve to twenty-four hours. In a concrete case 0.08 gram was used in the first injection without producing any vomiting, while a reinjection of only 0.001 gram gave rise to this at once. In other words, at the time of the second injection the animal was eighty times more

sensitive than before the first. Richet further showed that while the primary injection produces no material effect upon the blood pressure the second injection is followed by a marked drop.

Evidently then the first injection has in some manner called forth a hypersusceptibility to the special toxin, which in the present instance is characterized by an increased velocity as well as an increased intensity of reaction. This type of hypersensitiveness, Richet has termed *anaphylaxis*, indicating the absence of protection, in contradistinction to prophylaxis or immunity.

Arthus Phenomenon.—The following year (1903) Arthus then showed that similar results may be obtained with substances which unlike the actinocongestin are altogether non-toxic. For on injecting rabbits at definite intervals with normal horse serum, he found that the first two or three doses were promptly absorbed, but that subsequent injections led to increasingly more severe local reactions, so that at times gangrene even developed. This occurred no matter whether the injections were all made subcutaneously, or the first ones given intraperitoneally and only the last ones hypodermically. If the animals, moreover, were first injected subcutaneously, and subsequently intraperitoneally or intravenously, serious general disturbances (dyspnea, diarrhea, convulsions) and even death resulted (*Arthus phenomenon*). Corresponding results were obtained with milk, and Arthus could show that the anaphylactic reaction in question was specific, as an animal that had been sensitized with horse serum, for example, was not injured by the subsequent injection of either milk, white of egg, or the serum of other animals, but only of horse serum.

Early Studies of v. Pirquet.—Corresponding clinical studies were undertaken almost simultaneously by v. Pirquet, and were based upon the independent observation that a second injection of horse serum in a child was not followed by symptoms of serum sickness at the expiration of *ten* days, as had been noted after the first injection, but that they occurred in the course of the same day on which the second injection was given. He concluded that the then existing doctrine regarding the time of incubation in the different infectious diseases was erroneous, and propounded the hypothesis that the pathogenic agent calls forth symptoms of disease only after it has been changed by corresponding antibodies, and that the *period of incubation represents the interval of time which is necessary for*

antibody formation. Subsequently he showed in a joint publication with Schick that his original observation merely illustrated the general rule that a first injection of horse serum always sensitizes the individual to subsequent injections, so that the latter are followed by symptoms more rapidly and more uniformly and can be produced by doses which are much smaller than the first ones.

In contradistinction to Arthus who ascribed the hypersensitive-ness of his animals to the *repetition* of the injections in a general way, and who thought that it increased in intensity with each injection, v. Pirquet and Schick emphasized that a *single* injection suffices to bring about this result, and that a certain interval must elapse before the animal responds in the changed manner to the second injection. If, for example, the injection is repeated after five days, no induration develops at the site of the puncture, while at the expiration of ten days this is very marked. Subsequent injections usually lead to still more marked reactions, but v. Pirquet has shown that even then a diminution in susceptibility may occur, and that hypersensibility and immunity can accordingly not be separated in principle.

Theobald Smith Phenomenon.—Further experimental studies were then called forth by the observation of Theobald Smith that guinea-pigs which had once been used in the titration of diphtheria anti-toxin and which had hence been injected with a toxin-antitoxin mixture were thereafter hypersensitive to subsequent injections of horse serum. If such animals were reinjected they showed immediate symptoms of a serious character; they became restless, dyspneic, the heart action became feebler and feebler, the temperature dropped below the normal, and in fully 50 per cent. death occurred within a half hour (*Theobald Smith phenomenon*). Post mortem, a most striking picture was seen which readily explained the majority of the symptoms which preceded the fatal end, for on widely opening the thorax the lungs did not collapse, but remained rigid in a state of deepest inspiration. This phenomenon was first described by Auer and Lewis, and is attributed by these investigators to spasm of the smallest bronchioles, which virtually causes the suffocation of the animal.

At Ehrlich's suggestion, his pupil Otto took up the investigation of this problem and almost simultaneously with his report there appeared a detailed study of the same subject by Rosenau and

Anderson. From the experiments of these observers it appears that the toxin in itself has nothing to do with the sensitization of the animals, and that the horse serum produces this effect only if used in small doses, but that the toxin in some manner which is not yet understood facilitates the sensitizing influence of the serum. The small size of the dose of serum which in itself is sufficient to cause sensitization is indeed remarkable. In one instance Rosenau and Anderson produced this result with $\frac{1}{1000000}$ of a c.c., while amounts ranging from $\frac{1}{250}$ to $\frac{1}{1000}$ c.c. were sufficient in every instance; and while a first injection of 10 c.c., which is equivalent to 40 c.c. pro kilo of animal, caused no symptoms of any kind in the guinea-pig, 0.1 c.c. as second injection was sufficient to cause death.

Like Arthus they found the reaction to be specific insofar as it was impossible to produce symptoms in animals that had been sensitized with horse serum, by subsequently injecting them with the serum from animals of a different species. Like v. Pirquet, they could also show that a certain time interval must elapse between the two injections before anaphylactic symptoms develop, and that this depends to a certain extent upon the point at which the first injection is given; if this is made into the brain the animal becomes sensitive after the eighth day, while following a subcutaneous injection this occurs at least two days later.

Antianaphylaxis.—Especially interesting also was the discovery that if an animal is reinjected shortly before the twelfth day the reaction is only slight or may not occur at all, and that subsequent injections, for a certain time at least, produce no deleterious consequences; in other words, the animal has become resistant instead of hypersensitive (*antianaphylaxis*). This condition, however, is not permanent and after a number of weeks the animals gradually become hypersensitive again. If, however, the injections are repeated in increasing doses and properly spaced a state of resistance can be produced which lasts for many months.

The same condition may, however, also be brought about at a time when the animal is already hypersensitive, by injecting it with a sublethal dose of the antigen. The animal may then become violently ill, it is true, but it will recover, and is then markedly antianaphylactic, so that it can be injected even after a very brief interval with possibly a hundred fold larger dose of the

antigen without any deleterious results (see also Mechanism of Antianaphylaxis).

Anaphylactogens.—Subsequent investigations have then shown that an anaphylactic reaction can be called forth by the injection not only of blood serum, but also of milk, albuminous urine, sweat, bile, red cells, extracts of various normal tissues as well as of neoplasms, the contents of echinococcus cysts, extracts of lower animals or of vegetable organisms, including bacteria, etc., in short by any substance of albuminous character, and it is especially noteworthy that this in itself need not be toxic to the slightest degree. It seems, indeed, as though true toxins could not produce anaphylaxis, and that if this apparently occurs, it is due to contaminating albuminous substances. This, however, does not preclude the possibility that toxic albumins may give rise to the reaction, and we have seen, as a matter of fact, that Richet's original experiments were carried out with such material. In such an event, of course, the toxic character of the albumins may blur the picture somewhat; this is what actually occurred in the case of Richet's actinocongestin, and no doubt led to his assumption that the extract contained both a toxic (anaphylactic) substance and a non-toxic immunizing (prophylactic) principle.

Collectively those substances which are capable of rendering an animal anaphylactic are spoken of as *anaphylactogens*, *allergens*, or *sensibilisinogens*.

Serum Sickness.—It was next shown that any animal may be rendered anaphylactic, but that the mode and intensity of the reaction is not the same in all. The most susceptible animal is evidently the guinea-pig, and we have already seen the manner in which it reacts to the introduction of horse serum. In man the same antigen leads to those symptoms which collectively are spoken of as serum sickness, the most common of which are the occurrence of fever, of exanthemata, and of swelling of the joints. In dogs we note great restlessness, crying out aloud, and marked fall in blood-pressure, non-coagulability of the blood, and leukopenia. In goats extreme myositis has been observed.

Passive Anaphylaxis.—Most important further is the observation that the *anaphylactic reaction product* (the *anaphylactin* or sensibilisin of the French; the *allergen* of v. Pirquet) can be transferred from one animal to another, in a manner quite analogous to the

production of passive immunity, and writers hence speak of *passive anaphylaxis*, which may be homologous or heterologous, *i. e.*, it can be transferred to an animal of the same species or to one of a species which is different from the one which was actively sensitized. The discovery of this fact has had important bearings upon our understanding of the mechanism which underlies the production of the anaphylactic shock, for it showed conclusively that humoral factors are here at play.

Production of the Anaphylactic Shock.—Richet originally propounded the hypothesis that as a result of the first injection a special antibody is formed, which he termed *toxogenin*, and that this then splits off a highly toxic poison from the primary toxin, for example, from the anaphylactogenic principle of his actinocongestin. This hypothesis, however, cannot be applied to the anaphylactic reaction which follows the administration of non-toxic antigens, and is evidently based upon false premises. Other writers, such as Weichardt, v. Pirquet and Schick, Wolff-Eisner, Friedberger, Friedemann and Isaac, also assume the formation of antibodies, but suppose that a special toxin is set free from the corresponding non-toxic antigen when the two meet. Regarding the manner in which this occurs, different possibilities, of course, suggest themselves. Led by his observations on the anaphylactic reaction which follows the introduction of alien cells into the rabbit, Wolff-Eisner assumed a lytic action on the part of the anaphylactic antibody upon the corresponding albuminous antigen, analogous to the lytic action of the cytotoxins (*e. g.*, bacteriolysins) and a consequent liberation of endotoxin-like substances. Weichardt arrived at similar conclusions on the basis of analogous experiments with placental cells, but, unlike Wolff-Eisner, he assumed that the lytic action of the antibody does not set free preformed endotoxins, but that the lysis is followed by further chemical changes.

More recent investigations, notably by Dörr and Russ, have rendered it highly probable that the antibody in question is really a precipitin, and the predominating idea at present is that an anaphylactic toxin is in some manner split off from the corresponding precipitate through the agency of complement. This view is, as a matter of fact, supported by numerous observations. It has thus been shown that those split-products of the albumins which no longer give rise to precipitin formation, likewise do not act as sensibilisino-

gens, and that there does not exist a single precipitinogenic protein which has not also anaphylaxis-producing properties. Precipitating sera, moreover, always contain the anaphylactic-reaction product. Whether or not special albuminolytic amboceptors may also be concerned in the anaphylactic reaction must thus remain an open question. So much is certain that precipitin formation and anaphylactin formation evidently run a parallel course, and that there is no good reason for doubting their identity. This, at least, seems established for the anaphylactic reaction product which is formed as the result of the parenteral introduction of albumins. In the case of animal or vegetable *cells*, on the other hand, there is evidence to show that the cytolytic amboceptors may play the role of the anaphylactins.

Complement and Production of Anaphylactic Toxin.—That complement is necessary for the production of the anaphylactic toxin has been demonstrated beyond a doubt. Friedemann and Friedberger have thus shown that when fresh complement is added to a mixture of an albumin and its corresponding antiserum, in the test-tube, a toxic product (anaphylatoxin) is formed which, upon injection into a suitable animal, calls forth practically all the characteristic symptoms of anaphylaxis. Quite in accord with this observation is the fact that during the anaphylactic reaction, produced in the usual way, the complement of the blood is reduced to one-fifth or even to one-half of the original amount, and that the shock cannot be prevented by the artificial introduction from without of complement, even in large amount. Moreover, if an animal (such as the pigeon) which is biologically far removed from the rabbit, and whose complement does not supplement the action of any amboceptors formed in the latter, is injected with the serum from a sensitized animal of this order, and then reinjected with the corresponding antigen, no anaphylactic shock should theoretically develop, and, as matter of fact, does not develop. As in the test-tube experiment, furthermore, the action of complement can be prevented through the addition of a suitable amount of salt, *i. e.*, by raising the osmotic pressure of the mixture; so, also, is it possible to prevent the development of the anaphylactic shock in sensitized animals by a preliminary injection of large amounts of salt.

Nature of Anaphylactic Toxin.—Regarding the nature of the anaphylactic poison, our knowledge is as yet quite meager. If we

regard the action of the amboceptor-complement combination upon the albuminous antigen as comparable to the digestion of proteins by the digestive ferments of the gastro-intestinal tract, in other words, as a parenteral digestion, then we can also suppose that the anaphylactic poison represents some cleavage product of the protein molecule. As a matter of fact, there is a certain similarity of the symptoms of the anaphylactic shock in dogs to what we see in "peptone" poisoning in the same animal. In both instances there is a marked drop in blood pressure, incoagulability of the blood and leukopenia, and in both cases it is possible to counteract the poisonous effect by the administration of barium chloride. In other animals, however, such as the guinea-pig, "peptone" apparently plays little or no role; Witte peptone, indeed, is quite harmless for this animal, which, after all, is the most sensitive to anaphylactic shock. Barium chloride, moreover, does not prevent the latter, and a primary drop in blood pressure, such as we see in dogs, does not occur. But it is conceivable that while "peptone" does not play an important role, if indeed any, that other poisonous substances may be formed which may be quite harmless for the dog, but highly toxic for the guinea-pig.

Whether or not the anaphylactic poisons which are split off from different antigens by the antiserum of a given animal or animal species are identical is unknown, but does not seem unlikely in view of the uniformity of the anaphylactic symptom complex.

In speaking of the anaphylactic poison in the foregoing pages we have repeatedly made use of the term *anaphylatoxin*, which has come into common use so extensively that it would indeed be difficult to replace it. This term, of course, suggests that the poison actually belongs to the class of toxins which, as we have seen, are characterized by the fact that on immunization they give rise to a corresponding antitoxin. As yet there is no evidence, however, to show that it is possible to immunize against this poison, and it would accordingly be better not to use the term anaphylatoxin at all, or if so, to bear in mind that by "toxin" in this case we merely mean a poison in the more general sense of the word.

Seat of Interaction between Antigen and Antibody.—While in the past it seems to have been assumed by the majority of observers that the interaction between antigen and its anaphylactic antibody takes place in the circulation, evidence has of late been adduced

which suggests that the anaphylactic reaction may be brought about in consequence of an interaction between sessile antibodies, *i. e.*, antibodies which are still in union with the cells which gave rise to their formation, and the corresponding antigen. This view is supported by the experiments of Schultz and notably of Dale on the one hand, and of Manwaring, Voegtlin, and Bernheim on the other. Dale thus showed that the virgin uterus of sensitized guinea-pigs, after being freed from serum by thorough perfusion with Locke's solution, will exhibit a definite rise of tonus in response to extreme dilutions of the respective antigen, and that one dose of the specific antigen, in sufficient concentration to produce a "maximal" response of the anaphylactic plain muscle, completely desensitizes the latter to further doses of any dimensions. Corresponding results were obtained with the isolated lungs of the animal after perfusion with Ringer's solution. While in the guinea-pig the localization of the anaphylactic reaction in the plain muscle tissue has thus been established, Manwaring, Voegtlin, and Bernheim have conclusively demonstrated that in the dog the primary effect takes place in the liver. If the liver of a sensitized dog is thus excluded from the general circulation, the reinjection of the corresponding antigen does not produce shock; if, however, at this time the clamp on the hepatic artery is removed anaphylactic symptoms promptly develop.

While these experiments of course strongly suggest the existence of sessile anaphylactic antibodies in the tissues which have been studied, it does not follow that an interaction between antigen and antibody may not also occur in the circulation with consequent production of shock. That the anaphylactic antibody finds its way into the circulation can hardly be disputed, and one could conceive that under certain quantitative conditions it could here play the role of an antitoxin. This possibility has indeed not yet been satisfactorily worked out. At the same time one could also imagine that this combination is at first only a loose one and that it might yet be broken by the sessile antibodies with consequent anaphylactic shock. Opposed to the idea that the free antibody might play the role of an antitoxin, *i. e.*, of a protective substance, is the fact that as a consequence of the interaction between antigen and antibody in the test-tube, a reaction product develops which on injection into the normal, non-sensitized animal will develop anaphylactic symptoms.

Further work along these lines will be necessary before a true

picture of the proceedings can be developed, but the work of Schultz, Dale, Manwaring, Voegtlin, and Bernheim mark a most important advance and clearly show the direction in which profitable research may be pursued.

Mechanism of the Anaphylactic Shock.—If now we inquire into the mechanism by which the anaphylactic shock is called forth, the very suddenness of the onset and the lightning course of the reaction suggest a cerebral origin of the symptoms in question. As a matter of fact Besredka has shown that the shock, in guinea-pigs at least, is particularly severe if the anaphylactic poison is injected intracerebrally, and that it is then exceptional for an animal to escape death, while with the usual intraperitoneal method nearly 75 per cent. recover. Quite in accord with this view also is the observation that it is possible either to suppress or to mitigate the severity of the symptoms, if the animal is previously anesthetized with ether or ethyl chloride, or is treated with hypnotics, such as urethane, paraldehyde, or chloral hydrate.

Biedl and Kraus, on the other hand, working with dogs, came to the conclusion that primary injury to the brain can probably be excluded, since paralysis and respiratory disturbances were not observed and the reflexes did not disappear. They could note as a constant symptom, however, a marked drop in blood-pressure (from 120 to 150 Hgmn. to 80, 60, and even 40), which was shown to be of peripheral origin, and they are inclined to attribute practically all other symptoms, which have been noted during the anaphylactic reaction, including the drop in temperature which is seen in all cases, to this one factor. The effect of the narcotics and hypnotics they explain by the assumption that these remedies merely render the central nervous system less susceptible to the effect of stimuli resulting from the drop of blood-pressure and the consequent central anemia.

Auer and Lewis also assume a peripheral origin of the anaphylactic symptom complex, but regard the spasmodic contraction of the smallest bronchioles which is so constantly seen in guinea-pigs as the essential factor, in these animals at least. It would thus appear that in different animals the mechanism may be different, but the possibility must also be borne in mind that these differences may be more or less accidental and not essential. This idea is supported by the observation of Schultz and Jordan that the bron-

chial mucous membrane of guinea-pigs is especially thick and folded in such a manner that relatively slight contractions of the muscle fibers, which in other animals would lead to no untoward results, might be sufficient in the guinea-pig to effect complete occlusion of the bronchial lumen. Evidently, however, our knowledge of existing conditions is as yet too meager to warrant any far-reaching conclusions.

Mechanism of Antianaphylaxis.—As regards the mechanism which underlies the production of *antianaphylaxis*. Friedberger has suggested that it might be due to the absorption or neutralization (sc., inactivation) by the second dose of antigen of any antibody that may be present at the time, so that a subsequent injection of antigen does not meet with enough antibody to form a fatal dose of poison. Antianaphylaxis will hence be observed during the pre-susceptible stage, because not enough antibody has as yet been formed and during the susceptible stage (if a subfatal dose of antigen is injected), because all or most of the antibody is used up by the subfatal dose of the antigen. This principle is now practically utilized when the necessity arises of reinjecting a patient who has been sensitized by a previous injection of alien serum (antitoxin). The individual then receives a very small dose of the serum to be administered about an hour or two before the full dose is given, or the serum may be administered so slowly that the antianaphylactic state can actually develop during the injection (see Administration of Diphtheria Antitoxin).

Friedberger's explanation of the mechanism underlying the development of antianaphylaxis, as outlined above, is in perfect accord with the experimental facts, such as its abrupt development, its specificity and even with the *apparently* contradictory observation that anaphylactic susceptibility can be passively transferred to another animal even during the antianaphylactic state (as a portion of the anaphylactic antibody may have escaped neutralization). As Pfeiffer and Mita, moreover, have shown, the proteolytic ferment which may be demonstrated in the serum of the sensitized animal, disappears with the development of antianaphylaxis.

Strongly in support of Friedberger's view also is the fact that sensitized animals which have been rendered antianaphylactic with the homologous serum are thereby not protected against the anaphylatoxin itself.

Not to be confounded with the antianaphylactic state proper is the protection which may be afforded the sensitized animal by various other procedures, such as the injection of peptone, of heterologous alien sera, and even of indifferent inorganic substances like kaolin. Such protection in contradistinction to true antianaphylaxis is, however, not specific, does not develop abruptly, and rapidly disappears while true antianaphylaxis persists for a much longer time, and is within quantitative limits absolutely specific.

CHAPTER XI

ANAPHYLAXIS IN ITS RELATION TO DISEASE

THE discovery that the parenteral introduction of foreign albumins into the animal body leads to an anaphylactic state, in consequence of which the reintroduction of the corresponding substances is followed by changes which are of more or less serious effect upon the body at large, has, of course, raised the question, whether certain symptoms which we observe in the course of the various infectious diseases may not be anaphylactic in origin and whether certain non-infectious diseases may not be referable to such factors altogether.

A study of the various diseases from this standpoint has elicited a number of interesting data, though we must admit that our knowledge of these questions has not extended very far beyond the domain of possibilities.

The earliest investigations in this direction we owe to v. Pirquet and Schick, and from these we are unquestionably justified in inferring that the second possibility above mentioned actually exists, viz., that diseases occur which may be wholly due to the existence of an anaphylactic state in reference to certain proteins. The most notable example of this order is the *serum sickness* which is observed in certain individuals, following the injection of the various antitoxic and bacteriolytic sera, and which, as we have already pointed out, is not referable to the contained antibodies, but to the albumins of the alien sera in themselves. The picture which here develops is in many respects very similar to what we see in certain infectious diseases, although the material which is introduced is, of course, sterile. Here, as there, the clinical symptoms do not appear at once, but only after a certain interval, which is quite analogous to the so-called *period of incubation* of the infectious diseases.

This observation is very important, as it has thrown a new light upon the occurrences in the body during that period and upon the manner in which some of the clinical symptoms of the infectious

diseases may originate. In the past we have looked upon the "period of incubation" as representing a period of time during which the infecting organisms multiply in the body of the infected individual to that point at which they would be sufficiently numerous to give rise to symptoms of disease, either through their toxins (sc., endotoxins) or through interference with the metabolism of the macroorganism in other ways. This explanation, however, is manifestly out of the question in accounting for the "period of incubation" which precedes the development of the serum sickness where no infecting organisms are at work. But v. Pirquet has pointed out that the phenomenon is readily accounted for, if we bear in mind that during this period antibody formation is taking place, and that an antibody-antigen reaction will occur, as soon as the former has progressed to a certain point.

This point we may well term the *threshold of anaphylactic* or more generally speaking of *allergic reaction*. If at this time the antigen—in the present instance the albumins of the horse serum—has disappeared from the circulation, no symptoms will, of course, result; if, however, some of the material is still present, a reaction occurs, during which, as we now know, poisonous substances (anaphylatoxins, apotoxins) are produced, and to these in turn we may logically attribute the symptoms which then develop. The occurrences just described may be diagrammatically represented, as shown in Fig. 2.

If, following the first injection of horse serum, a certain interval be allowed to elapse, and a second injection be then given, the result will differ from the first not only in point of time of reaction, but also qualitatively and quantitatively, so far as the symptoms are concerned. If the second injection be given at a time when antibodies are still present in the circulation in considerable amount, a reaction will occur either immediately or within the first twenty-four hours; this may be quite violent in its intensity, though its duration is shorter than in the first instance. This *immediate reaction* is also diagrammatically represented in Fig. 2.

If, on the other hand, the second injection be given after several years, *i. e.*, at a time when the antibodies called forth by the first injection have disappeared, a certain interval of time will elapse before symptoms of serum sickness develop, as in the case of the first injection. But whereas this interval in the first instance is

usually from eight to twelve days we now find that symptoms appear after from four to seven days. Reactions of this order

FIG. 2

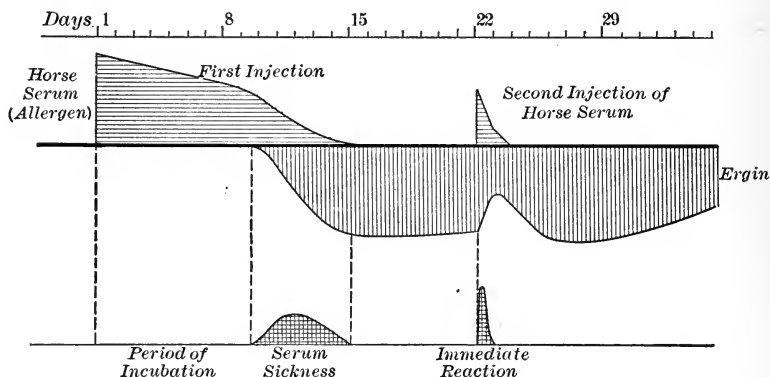


Diagram representing the interaction between horse serum and the corresponding ergin in relation to the development of serum sickness and the occurrence of an immediate reaction. (Taken from v. Pirquet.)

FIG. 3

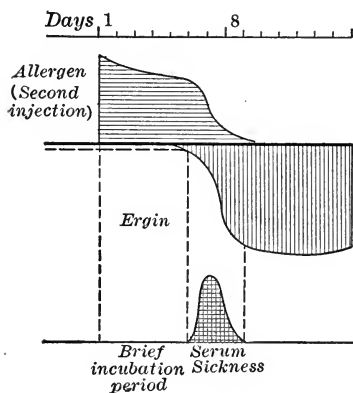


Diagram representing the interaction between allergen and the corresponding ergin in relation to the occurrence of a double reaction, i. e., an immediate followed by a hastened reaction. (Taken from v. Pirquet.)

FIG. 4

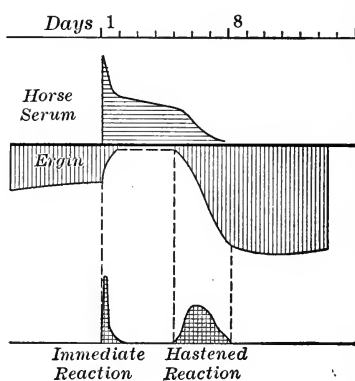


Diagram representing the interaction between horse serum and the corresponding ergin in relation to the occurrence of a hastened reaction. Note the greater depth of the antibody fraction as compared with the preceding figure. (Taken from v. Pirquet.)

v. Pirquet speaks of as *hastened reactions*. They are readily accounted for if we remember that a cell which has once been stimulated to active antibody formation (sc., liberation) will subsequently respond

to the same stimulus with increased activity. This may be diagrammatically represented, as shown in Fig. 3.

Theoretically we should expect another possibility to exist, viz., the occurrence of an *immediate, followed by a hastened reaction*, as the result of a second injection. This may actually occur, and is readily explained by the assumption that at the time of the second injection a small amount of antibody was still present, but that this was not sufficient to satisfy the affinities of the total amount of albumin introduced; that a portion of the latter hence calls forth the production of an additional amount of antibody which occurs in a "hastened" manner, and meeting with some of the free antigen gives rise to the hastened reaction, as shown in Fig. 4.

If now we compare these findings with certain occurrences which may be observed in connection with some of the infectious diseases, it will be seen that the appearance of certain symptoms which we note in some of the latter, may readily be explained upon the same basis as the reactions which follow reinjections of horse serum, as above outlined, so that the inference seems justifiable that the underlying mechanism in the two groups of cases is also essentially the same.

As is well known a first vaccination with cowpox lymph is followed by a period of seven or eight days, during which there is a slowly developing local reaction without any noticeable systemic symptoms. During the first two days the local response is evidently purely traumatic in character. On the third or fourth day the specific reaction begins in the form of a small papular elevation which, between the fourth and sixth days, is then differentiated into a central papilla and a surrounding areola. Up to the eighth day the latter extends but slightly beyond the papilla, but between this and the eleventh day it rapidly develops so as to form a well-marked inflammatory zone surrounding the central area, reaching its largest size between the eleventh and the fifteenth day. After this it gradually disappears, while the papilla dries up and exfoliates. Coincidentally with the development of the areola, there are frequently also systemic symptoms, of which fever and leukopenia are the most striking.

If now we compare this picture with that of serum sickness, we find very striking points of similarity which strongly suggest that the underlying mechanism is in all probability the same. Here, as there, we have a period of incubation of virtually the same duration.

But, whereas the injection of horse serum does not necessarily give rise to any symptoms during this time, since the material that is introduced is sterile, vaccination is early followed by certain local symptoms which we may logically attribute to a multiplication of the organism of cowpox in the skin. This is shown diagrammatically in Fig. 5 in the gradually ascending line representing the first vaccination. We may then suppose that the absorption of some dead organisms (dead when introduced or destroyed by the local defensive forces shortly after their introduction), *i. e.*, of their proteins,

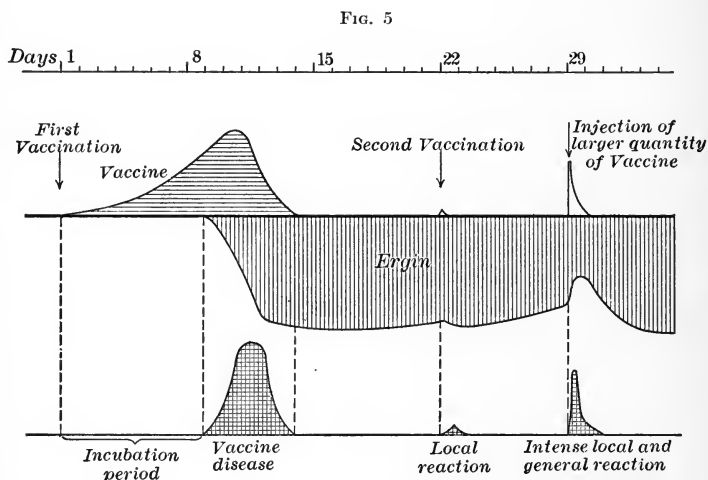


Diagram illustrating the effect of vaccination in its relation to antibody formation upon the development of the corresponding clinical symptoms. (Taken from v. Pirquet.)

is followed after the usual period of about eight days by the production of the corresponding antibodies, some of which, no doubt, bring about the destruction of all the remaining organisms, while others react with the liberated proteins and give rise to anaphylatoxins which in turn are responsible for the rapidly developing local inflammatory reaction, as also perhaps for some of the systemic symptoms. Theoretically, of course, the anaphylactic response should continue so long as both antigen and antibody are present, a conclusion with which clinical observation is in perfect accord. It might, of course, be argued that the period of incubation following vaccination was after all due to the multiplication of the variola organisms, and that so soon as this had exceeded a certain point, the local as well as the

general symptoms would have occurred irrespective of any antibody production. This conclusion, however, is disproved by the fact that the size of the vaccine dose neither hastens nor retards the development of the symptoms, and is further especially strikingly demonstrated in experiments of v. Pirquet, where the same patient was vaccinated on successive days. When this was done all points of vaccination developed their areola on the same day, so that in a given instance when the first vaccination reached its inflammatory maximum after eleven days the subsequent vaccinations were equally advanced after ten, eight, and four days.

In this connection it is interesting to note that whereas the vaccinated individual has acquired a marked immunity to infection with the organism of either human smallpox or cowpox, he is, nevertheless, hypersensitive to its proteins. v. Pirquet thus remarks that by frequently vaccinating himself he brought the skin of his forearm to such a state of hypersensitiveness that after twelve hours a papule, measuring on an average 9 mm. in diameter, *i. e.*, a size only reached in primary vaccinations on the seventh day, develops. This, of course, is closely analogous to what we see on reinjection with horse serum during the first six months following the primary injection, and represents what v. Pirquet terms a *hastened reaction*. An immediate reaction is here not observed, probably because it is obscured by the traumatic reaction.

If now we apply the same principle to the study of tuberculosis it will be seen that here also various phases of the disease can be satisfactorily explained upon the same basis (v. Pirquet). In experimental tuberculosis, produced in cattle, a quiescent period of "incubation," extending over eight days, likewise follows the inoculation, provided that the initial infecting dose was sufficiently large, exactly as in connection with vaccination and the development of primary serum sickness (Fig. 6). Coincidentally with the appearance of antibodies clinical symptoms then develop; but whereas in vaccination the production of antibodies leads to the prompt destruction of the invading organism, the tubercle bacilli, owing to their peculiar waxy envelope, no doubt succeed in maintaining themselves in the body of the infected organism, and may, if their initial number was sufficiently large, even cause the death of the host. A certain number, of course, are destroyed, and, as protein antigen and antibody thus coexist, a more or less continuous formation of anaphylatoxin

takes place, and becomes, in turn, to a certain extent at least, responsible for the more or less continuous symptomatic evidence of disease.

If the number of organisms is small, or if they have been attenuated by artificial means, the incubation period is much longer. In such an event the antibody production begins in the third week, but it is not until the fifth week that a sufficient quantity of anaphylatoxin is formed to elicit manifest symptoms (Fig. 7).

FIG. 6

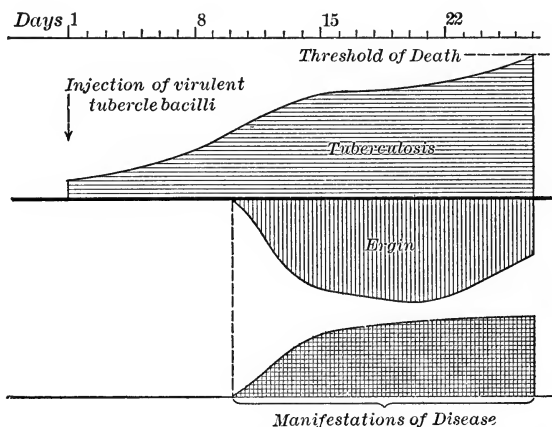


Diagram illustrating the interaction between antigen and antibody in a fatal case of cattle tuberculosis, following the injection of a moderate dose of tubercle bacilli. (Taken from v. Pirquet.)

FIG. 7

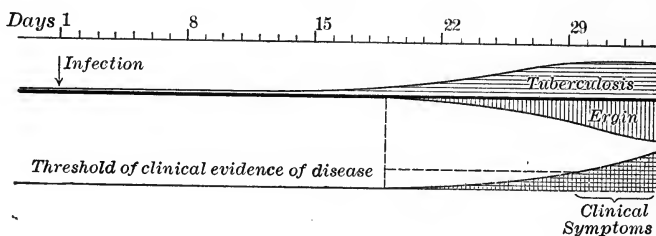


Diagram illustrating a protracted period of incubation in its relation to the interaction between tubercular antigen and the corresponding antibody; infection having been produced by the administration of tubercle bacilli in small number or in attenuated condition. (Taken from v. Pirquet.)

The subsequent course of the infection will, of course, depend upon circumstances. If recovery takes place the further multiplication of tubercle bacilli ceases; the foci that are already in existence are encapsulated and the active clinical symptoms disappear.

But, as is shown in Fig. 8, antibodies still remain in considerable amount, so that any factor which would now call a latent tubercular focus into renewed activity, or the introduction of tuberculin from without, would also call forth a prompt clinical reaction, either

FIG. 8

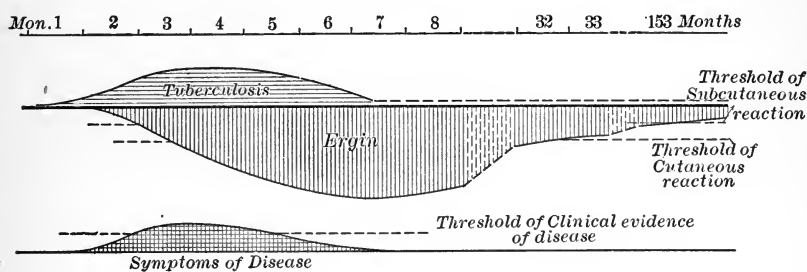


Diagram illustrating benign course in a case of human tuberculosis. (Taken from v. Pirquet.)

FIG. 9

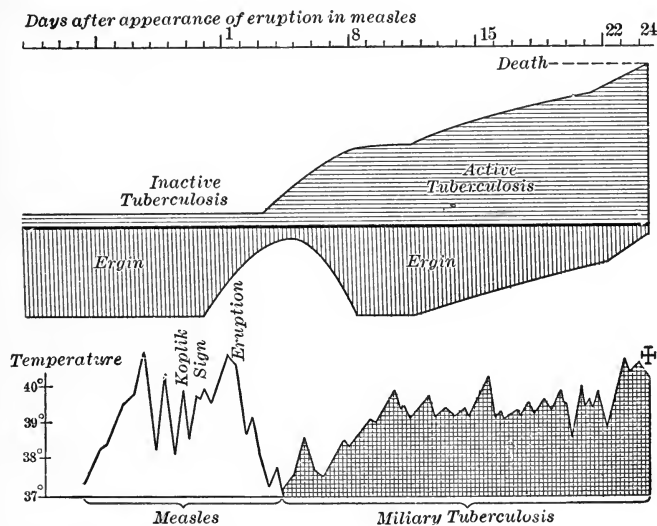


Diagram illustrating the lighting up of a tubercular process (miliary tuberculosis) following measles. (Taken from v. Pirquet.)

general or local, as the case may be. Fig. 9 illustrates this very well, and shows the mechanism which is called into action, when a miliary tuberculosis follows an attack of measles, in a subject having a latent (inactive) tubercular infection.

If now we pass on to a consideration of an infection with an organism which is a pure toxin producer the interpretation of the clinical picture will be different. In diphtheria, for example, we have coincidently with the multiplication of the invading organisms a production of toxin. This in itself is, of course, quite sufficient to account for practically all the clinical symptoms that we observe. These set in early, since comparatively few organisms are capable of producing toxin in sufficient amount to call forth clinical evidence of disease. There is hence not the usual incubation period of eight days, and the initial symptoms in any event are not due to any antigen-antibody reaction, but to the toxin itself. When the antibodies then appear we may, of course, rightfully assume that precipitins are formed, as well as lysins and antitoxins, and theoretically we might expect a clinical reaction due to anaphylatoxins. Clinically, however, we have no clear evidence of this, which is probably owing to the fact that the toxin effect by itself controls the entire picture. In scarlatina, v. Pirquet concedes that the primary malady, *i. e.*, the eruptive fever *per se*, is similarly a pure toxin effect, but that the sequelæ, and notably the nephritis, are the expression of the action of anaphylatoxins, which are formed, if at a time when the corresponding antibodies are present, an autoreinfection (from a broken-down lymph gland for example) occurs. A toxin effect, of the primary type, is then not produced, since antitoxins are at the time present in sufficient quantity to counteract their effect (Fig. 10).

It would, of course, lead too far to continue the analysis of the different infectious diseases along these lines, but I believe to have shown that the anaphylactic principle serves to explain many points in clinical symptomatology for which an adequate explanation has heretofore been lacking. If we consider that the absorption of alien proteins (and hence of bacterial proteins) probably always gives rise to the production of corresponding antibodies of the anaphyl-actin type, we can also understand that there is probably not a single infectious disease in which they are not formed, and in which they cannot, theoretically at least, play an active part. Besides the diseases already discussed this would certainly seem most likely in syphilis, in typhoid fever, measles, glanders, and pneumonia, and future studies of these diseases from this standpoint will, no doubt lead to interesting results. All along the line a start has indeed only

now been made, and a great deal remains to be done, but I believe that there is scarcely any field of study, which from the standpoint of the clinician promises such fruitful returns, as the investigation of our common infectious diseases and their pathogenic agents along these lines.

At the present state of our knowledge it is, of course, very difficult to decide which symptoms in a given disease are due to bacterial toxins, which to endotoxins, which to ptomaines, and which to anaphylatoxins; but it is important to recognize that with the discovery of the latter a new vista has been opened up, along which we can see the possible manner in which some of those organisms may produce

FIG. 10

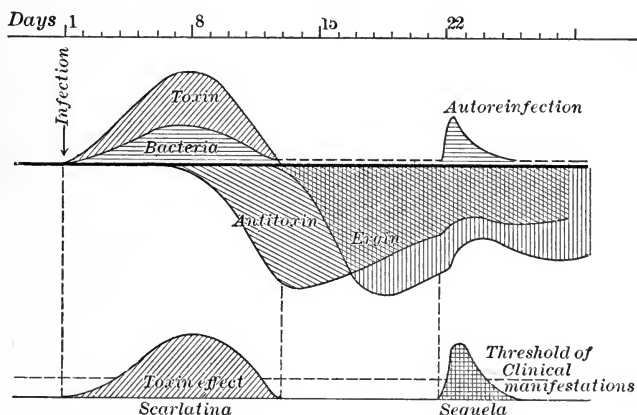


Diagram illustrating the interaction between antigens and antibodies in their relation to the clinical picture and a sequela of scarlatina. (Taken from v. Pirquet.)

symptoms of disease and even death, which are recognizedly not toxin producers, and whose endotoxins also are not sufficiently active to cause the clinically recognizable results of infection. This is indeed a most attractive field for speculation, but as a further discussion of the many possibilities and problems which suggest themselves in this connection would of necessity be purely theoretical in character, it will be better to leave this for some future occasion, when actual experimental data may be at our disposal.

Idiosyncrasies and Anaphylaxis.—I have pointed out above that the development of a definite symptom-complex following the parenteral introduction of horse serum (*i. e.*, of alien albumin) suggests the possibility that some of the non-infectious diseases with which we

have long been familiar may possibly be due to similar causes. Recent investigations have shown that such is actually the case, and with the recognition of this possibility an unexpected ray of light has reached one of the darkest corners of our clinical rubbish room where have reposed for centuries the time-honored and mystic "*idiosyncrasies*."

Especially interesting in this connection are the observations which have been made in the so-called "*hay fever*," or *pollen disease*, as it would be more appropriate to term the malady. As is well known, certain individuals are annually attacked with irritation of the mucosa of the nose, giving rise to paroxysms of sneezing, and later with a similar irritability of the pharynx and the trachea, leading to asthmatic disturbances of greater or less severity. The occurrence of these attacks is intimately associated with the time of the year at which certain plants (belonging to the order of the Gramineæ, also *Ambrosia* and *Solidago*) come into blossom, and is due, as Elliothson already pointed out in 1831, to the absorption of some constituent of the pollen of the respective plants. Weichardt and Wolff-Eisner then pointed out (1905 and 1906) the close similarity between the symptom-complex in question and the serum sickness of v. Pirquet and Schick, and suggested that *it* also could readily be explained upon the basis of anaphylaxis. As a matter of fact it is possible in a susceptible individual to call forth a typical attack at any time either by the introduction of a suspension of the corresponding pollen into the conjunctiva, or by its subcutaneous application, in a manner quite analogous to the tuberculin test. Here as there the amount of material which will suffice to bring about a reaction is remarkably small. Wolff-Eisner thus reports that a typical response will follow the application of but two drops of a 0.2 per cent. solution of pollen, and Lubbert mentions that in highly susceptible individuals $\frac{1}{40000}$ milligram even may produce symptoms.

Closely related to pollen fever are no doubt also those curious *asthmatic conditions* which have been noted in some individuals following the inhalation of Witte peptone, after the ingestion of egg albumen, strawberries, blueberries, gooseberries, various leguminous vegetables, lobster, chocolate, cheese, as also on exposure to certain exhalations, such as those of horses, in connection with attacks of constipation, etc. To the same category evidently belong also those curious cases of diarrhea which in some persons follow the

ingestion of egg albumen, and in certain babies the administration of cows' milk; further, also, the remarkable symptom-complex, which is usually designated as angioneurotic edema, the long list of urticarias which follow the ingestion of lobster, fish, oysters, cheese, and strawberries; certain bacterial exanthemata, certain skin affections of pregnancy, the dermatitis of satin-wood-workers, the phenomena of fagopyrismus (buckwheat poisoning), certain anomalous drug reactions, etc.

Here we have entered the very midst of the *idiosyncrasies* which in former years seemed shrouded in impenetrable mystery, and which now, in view of our knowledge of the principles underlying anaphylaxis, seem so readily accounted for on this basis, and as merely being the expression of an anomalous reaction on the part of certain individuals to the parenteral introduction of alien proteins. The question, of course, still remains to be answered why one person and not all others react in such an anomalous manner to stimuli which after all we must regard as normal. At the present we can merely theorize on these points, it is true, but we can do so with the knowledge that we have, at least, a basis which unquestionably is sound, and the time is evidently not far off when this chapter, which was only a few years ago so obscure, will be one of the best understood in physiological pathology.

This is not the place to enter into a detailed account of the various idiosyncrasies that we have just briefly passed in review, but it may be permissible nevertheless, before concluding our present chapter, to show by a few examples that we have already gained somewhat more than a clinical basis for our belief that anaphylactic action is responsible for the clinical pictures which we observe.

Especially interesting in this connection are the asthmatic phenomena which occur in some persons when exposed to certain exhalations. Remarkable examples of this order have been described. Schittenhelm thus mentions the case of an engineer who invariably was attacked with "asthma" when he was obliged to enter a tunnel that was under construction, while he was otherwise free from any discomfort. Another person was subject to asthma in one city and not in another only a few miles distant. To this order also belong those individuals who become asthmatic when they enter a horse-stable, or even when they sit in a carriage behind a horse. It is interesting to note that a number of persons who experienced a

severe anaphylactic attack immediately following the injection of horse serum were also affected by the exhalation from horses. All such cases are unquestionably anaphylactic in character and referable to the absorption of organic matter that is present in the emanations from animals and human beings. This explanation seems reasonable in view of certain findings reported by Weichardt. This observer noted that guinea-pigs which had been injected with fluid through which the expired air of human beings had been passed, were thus rendered anaphylactic to human bronchial secretion, and on intravenous injection with the latter responded with a marked drop in temperature and sopor.

Especially instructive also are those cases in which the ingestion of such common articles of food as egg albumen and cows' milk is followed by most abnormal consequences. Landmann thus records a case where the ingestion of a bit of egg albumen, no larger than a pea, was followed after a quarter of an hour by a burning sensation and swelling of the tongue, intense edema of the palate and pharynx, salivation, lacrymation, burning and itching in the Eustachian tubes, vomiting, severe diarrhea, and great prostration, while its application to the skin resulted in urticaria. Such findings are quite analogous to the anaphylactic enteritis which may be observed on injecting sensitized dogs with egg albumen, and where post mortem the mucosa and submucosa of the entire intestinal tract inclusive of the pylorus is studded with miliary hemorrhages.

In a case of so-called fagopyrismus (buckwheat poisoning), which unquestionably also belongs to this order, Thayer had the patient vaccinated with the substance in question, the result within half an hour being a feeling of oppression and nausea, frequent cough, a rapid and intermittent pulse, suffusion of the conjunctivæ, erythema, intense pruritus, and local urticaria at the point of injection.

In a case of antipork idiosyncrasy, Bruck injected some of the patient's serum into a guinea-pig and followed this up with an injection of hog serum, the result being a typical anaphylactic shock, while the control animals showed no symptoms whatever.

Quite recently the same writer has further shown that the curious hypersusceptibility which certain persons show toward iodoform can be passively transferred in the individual's serum to guinea-pigs, and Klausner could demonstrate that this was possible even after

an interval of fifteen months from the time of the last iodoform intoxication.

I myself, while suffering from a trichinous infection, developed a curious type of dyspnea, which continued for a number of months, and which I am now strongly tempted to look upon as an anaphylactic reaction due to the absorption of trichinous albumins.

Evidently this is a most interesting chapter in our modern immunity work, and one which is destined to assume an important position in clinical medicine.

CHAPTER XII

ACTIVE IMMUNIZATION

IF now we pass on to a discussion of the different defensive factors of the animal body from the standpoint of prophylactic and curative therapeutics the question naturally arises, To what extent have we the power to influence this mechanism artificially? In view of the fact that we have scarcely passed farther than the threshold of the study of immunology, using the term in its widest sense, it is natural that our attempts to utilize principles with which we have thus far become acquainted should have been relatively crude, and that the results in many instances have not led to a satisfactory end. An enormous amount of work still remains to be done, but even so we have every reason to be proud of what has already been achieved, and to believe that more yet will be accomplished in the future.

We have seen that even under normal conditions the body has at its disposal defensive forces which are most important, and which in many instances are quite sufficient to prevent a general infection, even though the local barriers have fallen. The battle here is, no doubt, frequently won before specific antibody formation—our second line of defence—has even begun. For many centuries physicians have recognized the existence of so-called predisposing causes to disease and their influence upon the course of the individual case. I would recall the effect of depressing influences, such as grief and worry, fatigue and hunger, in increasing the predisposition to a great many infectious diseases.

Quite in accord with clinical observation are the results of the animal experiment. Charrin and Roger thus succeeded in infecting rats with anthrax after they had been greatly fatigued by being made to run in a tread-mill, while under normal conditions the animals are quite resistant. Other observers could break the natural immunity of dogs, chickens, and pigeons to the same organism by the withdrawal of drinking water; in pigeons the same result can be obtained by fasting. Quite well known further, both clinically

and experimentally, is the predisposing influence to infection of the continued use of alcohol and various narcotics. Of the manner in which these agencies bring about the greater susceptibility to infection nothing was known in the past, and even now our knowledge is but imperfect. But we can at least suggest certain possibilities. As we know that phagocytic action represents one of the most important factors in our first line of defence, and as this has been shown to depend to a very great extent upon the presence of opsonins and tropins, it would seem reasonable to suppose that the harmful agencies just referred to might readily operate through interference with the production of such bodies as are essential to phagocytosis.

In this connection it is interesting to note that during pregnancy which has long been recognized as a factor predisposing to the development of tuberculosis the opsonic content of the blood tends to be abnormally low in fully 50 per cent. of the cases. Then, again, we can conceive that the normal bacteriolytic power of the blood may be impaired by some of the influences in question. In the case of chronic alcoholism, this has indeed been demonstrated by Abbot and Bergey, who noted that there was a diminution of complement.

That this in turn may actually diminish the resistance to certain infections has been shown by Pfeiffer and Moreschi. These investigators injected a series of guinea-pigs intraperitoneally with a fatal dose of cholera vibrios (equal for all animals) and an amount of cholera immune serum sufficient to protect the animals against the number of organisms used. At the same time they received varying amounts of normal human serum and a constant quantity of an anti-human rabbit serum. The latter, of course, contained precipitins for the human albumins, and the idea of the experiment was that as a consequence of the interaction between precipitin and precipitinogen (albumin), and the resultant formation of a precipitate the complement of the guinea-pig would be absorbed, and accordingly not be available to activate the anticholera amboceptors, so that the animal would lose the protective influence of the latter which would have been operative had complement been available. The results were quite in accord with the theoretical demands, all those animals having died in which occasion for complement elimination was afforded, while the control animals which had received no human serum, but which had otherwise been treated

in the same manner, lived. Evidently a lack of complement in the course of an infection may lead to disastrous consequences, and the assumption seems justifiable that the presence of an insufficient quantity at the start may favor the generalization of an infection in which otherwise a local reaction only might have taken place.

That the normal amboceptors might be similarly influenced is, of course, also possible, and is suggested by certain experiments of Abbot and Bergey, who found that the hemolytic amboceptors which appear in the guinea-pig following the injection of alien red corpuscles rapidly disappear under the continued administration of alcohol.

These data, few as they are, throw some light upon the possible *modus operandi* of some of the causes which *predispose* to infectious diseases, and open up a field for investigation which, speaking *a priori*, should furnish some very valuable results. Studies in this direction would also show by what general non-specific measures, dietetic, medicinal, or otherwise, the resistance against infection could be *raised*, and the likelihood of successful *specific* treatment thereby enhanced. At the present time the latter occupies the foreground of medical interest, and it is the purpose of the following pages to show what has already been accomplished, both from the standpoint of prophylaxis and of treatment.

In arranging the sequence of our subject matter, precedence is given to those methods by which immunity can be *actively* produced, for here the entire defensive mechanism is thrown into operation, whereas in the production of passive immunity only certain individual defensive principles are utilized.

(4) ACTIVE IMMUNIZATION FOR PROPHYLACTIC PURPOSES

Since the entire defensive mechanism of the animal body is thrown into action as a result of *active* immunization it would suggest itself that attempts in this direction would furnish the most valuable results when employed for prophylactic purposes. When infection has once taken place, and clinical symptoms of disease have already developed, conditions are much more complicated. The effect of toxins, whether produced by the infecting organisms themselves, or as a result of anaphylactic reaction, then so frequently dominates

the clinical picture that sufficient time is not available to stimulate the body cells to active immunization. If the period of incubation of a given malady is sufficiently long, so as to permit of the active mobilization of the defensive forces before symptoms of disease actually develop, attempts at active immunization would, of course, be indicated, and, as shown in our prophylactic treatment of rabies, may give rise to excellent results. Chronic infections further would theoretically, at least, lend themselves to treatment of this order, while in the acute maladies, for the reasons just indicated, we can only exceptionally hope to exercise a favorable influence upon the course of the disease. In combination with the use of antitoxic or bacteriolytic sera, however, it might even then be tried.

As the basis of all attempts at active immunization, we might very appropriately take the dictum: *that there can be no protection without infection*, bearing in mind, however, that "infection" is not synonymous with "disease," that "infection" does not imply a "virulent" infection, and that, immunologically speaking, the parenteral introduction of the *killed* pathogenic agent even may be equivalent in its effects to infection. That infection with living virulent organisms may result in protection has, of course, been recognized as long as we have had knowledge of the etiologic connection of microorganisms with the infectious diseases, but the discovery that the same result can be achieved in many instances without the production of any malady of moment, through the use of organisms whose virulence has been artificially diminished, and, as I have already indicated, even with organisms that are dead, is one of the greatest triumphs of modern medicine. The various methods that are employed to this end have already been briefly considered in a previous chapter, and will be taken up in greater detail in connection with the different infections against which active immunization is employed.

SMALLPOX

It is an interesting coincidence that the principle just stated, viz., the possibility of producing active immunity by the use of organisms that have been attenuated in their virulence, was unconsciously employed by the earliest workers in this field.

When and how the discovery was made that the virulence of small-

pox is greatly diminished by the introduction of the virus through the skin is not known. But the principle was evidently already extensively utilized in Turkey for prophylactic purposes early in the eighteenth century. For in 1718 Lady Montagu, the wife of the English ambassador at the Ottoman court, wrote to a friend as follows: "The smallpox so fatal and general amongst us, is here entirely harmless by the invention of *engrafting* which is the term they give it. Every year thousands undergo the operation, and the French ambassador says, pleasantly, that they take the smallpox here by diversion, as they take the waters in other countries. There is no example of anyone who has died in it, and you may well believe I am satisfied of the safety of the experiment, since I intend to try it on my dear little son. I am patriot enough to take pains to bring this useful invention into fashion in England."

As a matter of fact Lady Montagu's daughter was the first person inoculated for prophylactic purposes in England. The material used for this purpose was the purulent matter obtained from smallpox pustules "of the distinct kind," which was then applied to two small incisions just through the skin, on "Dossils of Lint."

Of the subsequent occurrences, Dr. Allen, a fellow of the Royal Society, then gives the following account: "About the eighth day after the operation some Pustules begin to appear, not unlike to those that are commonly seen in the distinct kind, a little Fever having preceded the Eruption, and the other usual Symptoms, but more mild and gentle. . . . In the general it is observable that the Smallpox procured by Inoculation are of the distinct kind, for the most part void of danger, that the Pustules are few in number and pit very little." With this method many thousands of persons were subsequently treated.

As regards the prophylactic value of these inoculations in England accurate statistical reports are unfortunately lacking, but it seems from the writings of contemporary observers that the protection was regarded as complete. As regards the dangers of the process there is some diversity of opinion. The Sutton brothers, who did a great deal to perfect the technique of inoculation, thus claim to have inoculated not less than 20,000 persons without losing one as the direct result of the operation. Dr. Gregory, of the London Smallpox Hospital, placed the mortality rate at one in five hundred. Sir Thomas Watson writes: "No doubt the distemper was produced

artificially in many more persons than would have caught it naturally, had inoculation never been thought of. So that while the relative mortality, *i. e.*, the percentage of deaths from smallpox, was lessened by this practice, the absolute mortality was fearfully increased."

It was noticed, moreover, that, contrary to expectation, persons who had been variolated occasionally themselves became centres of infection. Evidently the attenuation of the organism by skin passage was not always sufficient to make variolation an altogether harmless procedure. The underlying principle, however, is evidently sound, and for all ages to come these early attempts at protective inoculation will form one of the great turning points in the history of medicine.

The next step in advance is intimately associated with the name of Jenner. Led by the popular belief which was prevalent in Gloucestershire during the latter half of the eighteenth century, that individuals who have accidentally become infected with cowpox were thereby protected against smallpox, Jenner actually put this idea to the test (1796).

To this end he inoculated a healthy boy, of eight years, with material taken from a cowpox vesicle on the hand of a dairy maid, and a couple of months later showed by inoculation with smallpox virus that the child was actually immune. In 1798 he furnished further proof that cowpox will furnish protection against smallpox by inoculating, or, as we may now say, *vaccinating* (from *vacca*, the cow), a child directly from a cowpox vesicle, and continuing the inoculation from arm to arm through a series of five children, after which all five were variolated, *i. e.*, inoculated with human smallpox, the result again being negative.

Hereafter vaccination was extensively practised in different European countries, and also introduced in America, the source of material for a long time being lymph which was obtained from cows that had developed cowpox, and in some instances from horses, affected with *grease*, the affections having been shown to be identical. While Jenner and many later investigators failed to recognize the identity of human smallpox with cowpox, as well as grease, this seems now to have been satisfactorily established, the apparent differences between the two conditions and the effect of the inoculation of the two kinds of virus being the result of the attenuation of the organism in question, in consequence of animal passage.

While in former years vaccination was frequently performed by direct transmission from arm to arm, this has now been entirely abandoned, animal lymph being exclusively used. This is prepared in special laboratories, and put up in such form that the practitioner can carry out the vaccination at any place, whereas in former years the persons who were to be vaccinated were often obliged to come to the stables in which the animals that furnished the lymph were kept.

Preparation of the Vaccine.—The technique employed in the preparation of the vaccine is in brief the following (method in use at the Government Vaccine Institute of Vienna). The animals used are young cattle, not older than two years or younger than six months, whose freedom from disease has been previously ascertained. After being placed on the operating table the abdomen up to the umbilicus, as also the portion of the inner surface of the thighs, is shaved, the skin cleansed with green soap and water, then copiously rinsed with a 2 per cent. lysol solution, then with sterile water, and finally dried with sterile gauze. The entire surface is then scarified in longitudinal or transverse streaks, measuring about 10 cm. in length, and from 2 to 2.5 cm. apart, care being taken that the papillary layer is just barely touched, so that there is no bleeding. The virus is then introduced into these streaks, either by making use of a special vaccine lancet (*Chalybaeus lancet*) or by rubbing it in with a suitable instrument.

In Vienna, where so-called *retrovaccination lymph* is exclusively prepared, calf lymph is first inoculated into a healthy child, when lymph from this source is employed to inoculate the new animal; the resultant material is termed retrovaccine of the first generation. This can then be used for human vaccination, or, still better, the product obtained with it from a second animal—the so-called retrovaccine of the second generation.

After the animal has been prepared, as just described, the entire vaccinated surface is suitably protected against dirt and infection, and the animal returned to its stable, which is kept scrupulously clean. The result is seen in Fig. 11, which represents the appearance of the “pox” at the end of five days. At this time, or after three to four days in younger animals, the covering is removed, the entire surface cleansed, as described before, but not dried, when with the aid of a stout curette the surface material is scraped off, care being

taken that it is not contaminated with blood. With practice the vesiculated epithelium can be removed in long strips. The material is placed in a suitable receptacle, weighed and treated with five times its amount of sterile glycerin-water (80 parts of glycerin and 20 of water). The quantity which can usually be obtained from one animal varies between 25 and 50 grams (in the case of retrovaccine of the second generation). Twenty-four hours after the removal of the material the animal is killed, and if no disease that could

FIG. 11



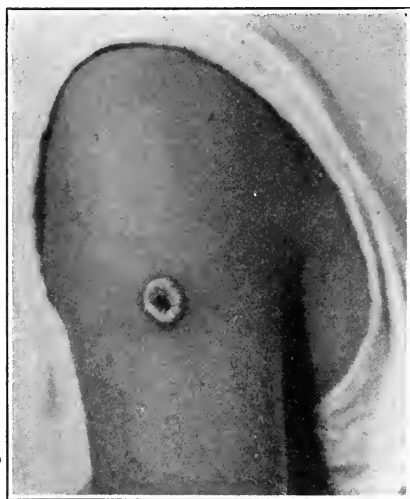
Belly of heifer, showing one of the approved modern methods of propagating vaccine virus; lesions photographed at the end of five days. (Taken from Welch and Schamberg.)

affect the vaccine is found post mortem, this is further treated as follows: After standing for four weeks in contact with the glycerin the mixture is thoroughly triturated in a "lymph mill," when the resultant emulsion is filtered through gauze and is then stored for at least three or four weeks at a temperature of 8°C ., the idea being to favor the destruction by the glycerin of contaminating micro-organisms, the admixture of which is practically unavoidable, even though the field of operation be ever so carefully protected.

If a bacteriological examination then shows the presence of but

few (*e. g.*, less than 30 per 0.01 c.c.) and the absence of all suspicious organisms, including the tetanus bacillus (the latter point is tested by injecting a mouse with 0.01 c.c.), the lymph is placed in capillary tubes, which are sealed at the ends, and is then ready for use. As the activity of the vaccine diminishes in the course of time, each preparation bears on its label the date, after which it should no longer be employed.

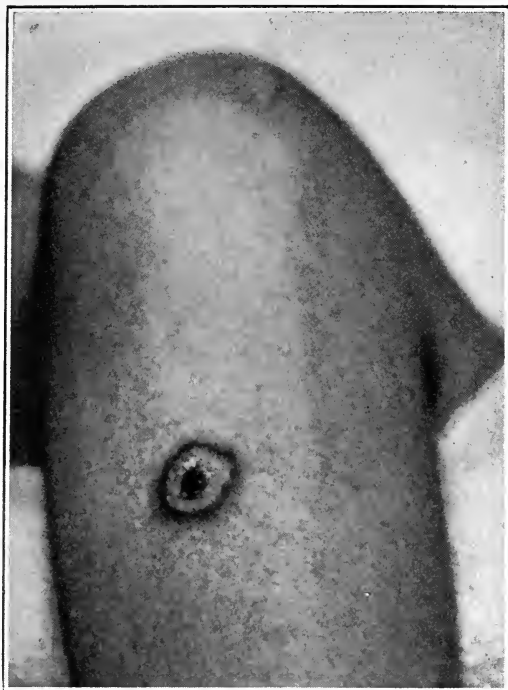
FIG. 12



Vaccine vesicle upon the seventh day; areola just beginning. (Taken from Welch and Schamberg.)

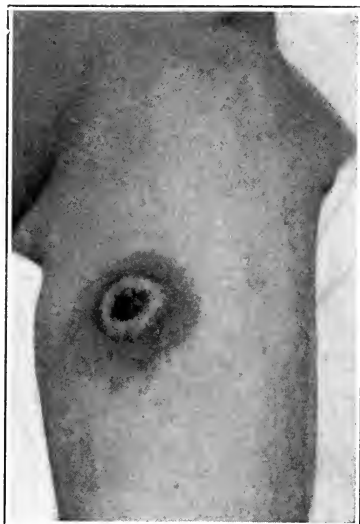
The Process of Vaccination.—The field of vaccination, which is preferably the upper portion of the upper arm, is first cleansed with soap and water, and then with alcohol or ether. With a suitable instrument, which must be previously sterilized, two or three parallel scratches are then made, about $\frac{1}{2}$ cm. in length and 3 cm. apart. A stout needle answers all purposes, and can be sterilized by flaming the point and then cooling it in alcohol. If desired, a new needle can be used for each case. Care should be taken that the scratch extends to, but not through the papillary layer; actual bleeding is to be avoided. The needle can either be charged with the lymph from the start, and the scratches made with the vaccine point, or a drop of the material is placed upon the scarified area and subsequently rubbed into the little grooves with the body of the needle. The removal of the lymph from the tubes is accomplished

FIG. 13



Vaccine vesicle upon seventh day, showing beginning areola. Patient was suffering from scarlet fever. Vesicle shows some irregularity in form. (Taken from Welch and Schamberg.)

FIG. 14



Vaccine vesicle upon ninth day, showing more pronounced areola. Same patient as Fig. 13. (Taken from Welch and Schamberg.)

by the aid of the tiny rubber nipple, which is sent out by most of the manufacturers with each set of tubes. When the vaccination is completed the arm is usually left exposed until the lymph has dried, so far as this is possible in the presence of glycerin. In Vienna it is customary to cover each scarification with a so-called tegmin dressing, which may be removed the following day or the day after. Subsequently the entire area may be dusted once or twice a day with a powder composed of 10 grams each of oxide of zinc and starch and 40 parts of talcum. This, however, is not necessary.

The appearance of the arm illustrating the results of a typical vaccination is shown in the accompanying illustrations (Figs. 12, 13, and 14).

The Protective Value of Vaccination.—This is now so generally recognized that it scarcely seems worth while to enter into a discussion of the question. Smallpox, which up to the time of Jenner was one of the worst scourges of the civilized world, has now become so rare a disease in those countries where vaccination is thoroughly carried out that the majority of physicians and medical students have not seen even a single instance of the disease. In Berlin, where the annual death-rate from smallpox before the introduction of vaccination varied between 250 and 400 per 100,000 inhabitants, the aggregate death-rate from the disease in entire Germany, even including imported cases, is now less than 0.1 per 100,000. An excellent idea of what systematic vaccination can accomplish may also be formed from the accompanying table, which indicates both the morbidity and mortality from smallpox in the German army, as contrasted with the results in the armies of Austria, France, and Italy, in which no systematic vaccination had been attempted:

Army.	Period.	Morbidity (No. of cases).	Average per year.	Mortality (No. of cases).	Average per year.
German	1875 to 1892	13	0.7	1	0.05
Austrian	1875 to 1886	9864	896.7	595	54.0
French	1875 to 1892	8356	491.5	705	41.4
Italian	1875 to 1894	2565	135.0	193	10.1

The same point is also well illustrated by comparing the number of deaths during the Franco-Prussian War in the entire German army—459, with that in the French—24,469.

In the face of such evidence a country that will not or cannot enforce systematic vaccination evidently courts the disease, and hardly merits a place in the rank of civilized nations.

RABIES

While the actual principle underlying the preventive vaccination against smallpox was scarcely recognized by Jenner and his contemporaries, their work nevertheless constitutes the basis of all our modern vaccine work, and to it may be directly attributed the successful preventive treatment of another dreadful disease, the pathogenic agent of which has likewise not yet been isolated, viz., rabies. This discovery we owe to the genius of Pasteur, and to him undoubtedly belongs the credit for having first recognized that by the use of suitably attenuated virus full protection may be afforded against the corresponding full-virulent infection. In Jenner's case nature had performed the experiment for him; but Pasteur was the first who purposely employed the animal experiment to demonstrate the principle in question.

The idea underlying Pasteur's antirabic treatment is to immunize the bitten individual within the period of time that the actual disease requires for its development. To accomplish this it was necessary so to change the nature of the virus that the incubation period following its injection should be materially shorter than that of the actual disease, which is usually from two to three weeks, but may be as long as two months, or even longer.

This was accomplished by passing the natural virus, or *street virus*, as it is usually termed, through a series of fifty rabbits, when its period of incubation was found to be reduced to but six to eight days. Further passage does not change this, and such virus, which, moreover, no longer produces the symptoms of furious rabies in dogs or guinea-pigs, but merely the paralytic type of the disease, is now termed *virus fixe*. In a certain sense, viz., insofar as the effect of the animal passage upon the period of incubation is concerned, we may look upon the virus fixe as being increased in virulence, but so far as its pathogenic properties go there is reason to think that for man this is actually diminished. Pasteur then found that the virulence of the virus in question can be still further diminished by desiccation, and that after twelve to fourteen days it is lost altogether. The plan of treatment then is to inoculate the patient

on successive days with material of increasing virulence, beginning with that which is altogether innocuous, *i. e.*, twelve to fourteen days old.

The technique employed in the preparation of the virus and the immunization of the patient, as described below, is that in use at the Pasteur Institute of the College of Physicians and Surgeons of Baltimore, under the direction of Dr. N. G. Keirle, and represents the original Pasteur method.

Preparation of the Virus.—The original virus was obtained from the Pasteur Institute of Paris, and had been started from the medulla of a rabid cow in 1882. It has since been transferred from rabbit to rabbit, and has now reached about the nine hundredth remove. Another virus was started by Dr. Keirle himself from the medulla of a rabid cow, and has reached about the six hundredth remove. As a rabbit will live about twelve days after inoculation, about thirty passages may be made in a year.

The inoculations are made as follows: "The hair between a line drawn transversely through the ears and eyes is cut off with scissors and washed with a 3 per cent. solution of carbolic acid. No anesthetic is required; the animal does not cry out, and evinces no sign of pain. The animal need not be strapped down, but may be held on the table by an assistant. A cut one inch and a quarter long is made with the knife or scissors, longitudinally, through the skin in the middle of the space at the top of the head between the lines above named. A blepharostat keeps the incision apart, and the sublying tissue is scraped away so as to expose the bone a little to one side of the median line. The trephine has a diameter of 5 mm. and a ring guard which is set at 2 mm. from the cutting end of the crown; the trephine may be a bit fastened in a revolving drill handle, or a simple hand trephine made of metal rod 17 cm. long. The button of bone is removed with a tenaculum and the dura is exposed, and an ordinary hypodermic syringe is used to inject three or four drops of the rabic emulsion beneath the dura. If the perforating end of the needle is curved almost at a right angle for a space of 4 mm. it facilitates its introduction, but this is by no means indispensable. The wound is closed by interrupted sutures (three are generally sufficient) and then sealed with collodion. The ordinary suture needle can be used, but the risk of sticking the hand is lessened if the needle has a fixed handle, the other extremity terminating in a spear with a slit in its side,

which is opened and closed by pushing a button. It is passed through the skin closed, then opened, the thread inserted, when it is again closed and withdrawn. Of course, all the materials and instruments have been sterilized, and during the operation are placed in a pan of 3 per cent. carbolic acid. The rabbit is then placed in a box properly labelled. Wire cages are generally used, but if the floor be of asphalt or of cement, a box without a bottom, having a wire grating for a lid, with a bed of sawdust or straw, is more convenient to keep clean.

"In an institute like the one at Baltimore, where approximately 120 cases are treated annually, two animals are daily inoculated, the material for this purpose being obtained from the medulla of those animals which have died of rabies during the day or the night preceding. To this end a piece from the floor of the fourth ventricle, measuring about 2 cm., in length is rubbed up in 1 c.c. of bouillon, and of this emulsion, as I have just stated, three or four drops are injected beneath the dura.

"As I have mentioned before, rabbits that have been inoculated with virus fixe develop rabies after an incubation period of from six to eight days (the shortest period being usually only reached after ninety passages), and then die almost four days later, viz., after ten to twelve days following the inoculation. The dead animals, as soon after death as possible, are sprayed with lysol or bichloride and stripped of their fur, when the cord and brain are removed under aseptic precautions. The cord is severed just below the medulla and divided into two equal pieces, which are suspended by sterilized silk threads in a sterile glass jar (aspiration or irrigation bottles, 1 liter capacity), the bottom of which has been covered about 2 cm. deep with flake caustic potash. The threads are held in position by the cotton stopper and are allowed to hang outside. The medulla is kept in a sterile dish and is used to continue the series of inoculations, as indicated above. The jars are labelled with the date, the number of the passage, and the number of the animal passage. A post mortem finally is performed and any cord rejected in which the animal is found diseased, or in which bacteriological examination of the cord has shown the presence of pyogenic organisms.¹ The jars are then kept in the cord room (occupying about 12 to 15 square feet) at a temperature of from 20° to 25° C.

¹ To this end a small piece is snipped off after twenty-four hours, dropped into bouillon, and this incubated until the next day.

"In preparing the emulsion with which to inject patients it is better to use the main room, and have a table with a drawer in which are labels, glass rods, curved scissors, and forceps, the glass rods having been wrapped in paper and sterilized in hot air. Erlenmeyer flasks, 100 c.c. capacity, with cotton plugs, contain water, sterilized in the autoclave. There are also stout wine-glasses covered with paper caps and sterilized in hot air; the wine-glass inside does not taper to a point, but has a flat bottom 2 cm. in diameter, which corresponds to the diameter of the glass rods, which are 25 cm. long; the wine-glass brimful holds 40 c.c. There is, of course, a Bunsen burner on the table.

"For the newly arrived patient the cord of the thirteenth and fourteenth day is used; that is, a cord that has been drying over caustic potash for thirteen and fourteen days. Such a cord measures transversely $\frac{1}{2}$ cm. The cord is for one instant passed into the Bunsen flame, then with curved scissors, previously heated to redness in the flame and allowed to cool, less than a millimeter, is transversely cut from the end of the cord and allowed to drop into the wine-glass; ten such pieces measure 8 mm. and weigh 20 mgm. This is triturated with the glass rod until it has become thoroughly broken or mashed according as it is recent or old. Then a few drops of water are added and it is triturated until a milky fluid results, then more water added until finally 15 c.c. is reached.

"The emulsion now looks like rice water, and a sediment soon accumulates; the paper cap is put back on the wine-glass, and on the bottom rim, upper surface, is put a label with No. 14 on it, meaning a cord that has dried fourteen days (older cords, 15-day cords, are rejected and the bottle cleaned). The caustic potash, after twice using, requires renewal; in twenty-eight days it looks like wet white candy. The glass rods wear smooth and require to have pieces cut with a file and broken off, thus becoming sharp again. Similar to the above, the thirteenth-day cord is prepared in a wine-glass. These wine-glasses are put in an agate-ware tray or baking pan in which are placed pieces of filter paper, and a bowl with some 3 per cent. solution of carbolic acid and two Pravaz syringes. A list is made out with the names of the patients and the age of the cords—that is, the number of days they have been drying, and the day of the month corresponding, which is the date on the bottle, and the dose, *e. g.*:

FOR JUNE 24, 1898

	Cord.	Date.	Dose.
George Williams	10 days	June 14	3 c.c.
Edward Cook	5 days	June 19	2 c.c.

"These lists are made out and put on the table the day before the treatment, and are entered in the case book, which records the circumstances of the patient's case, *e. g.*, name, age, seat of bite, number of bites, animal that inflicted bites, cauterized or not, what has become of the animal, etc.

"The temperature of the cord room and of the outside atmosphere, and the results of the culture are recorded in a book on the table in the main room. We now take the tray and go to the patient.

Treatment of the Patient.—"The patient is permitted to stand, sit, or lie down, as he or she may desire. The Pravaz syringes and needles, which have been filled with 3 per cent. carbolic acid solution, are emptied and washed out with sterilized water. These syringes, holding 3 c.c., are filled by thrusting a needle through the paper cap of the wine-glass; then the abdominal region of the patient is bared and the site of the injection (hypochondria, or anywhere on abdomen), avoiding large veins, is wiped with filter paper wet with 3 per cent. carbolic acid solution. Then the skin is raised in a fold between the fingers and the needle is thrust well into the subcutaneous tissue. It is important to avoid injecting the layers of the skin, which is painful, and to avoid sites of previous injection. After the injection a piece of filter paper wet with the carbolic acid solution is put on the skin and allowed to remain for a few seconds.

"We have not modified the dose relative to age. In our youngest patient, a girl of two and a half years old, and an old lady, aged eighty-four years, the same doses were given. At times there are redness and induration in the connective tissue, but there has never been pus, never cellulitis of the slightest gravity. Hot-water applications on towels suffice to remove any trivial inconveniences. The treatment occupies twenty-one days at least.

"First day (thirteenth- and fourteenth-day cord) 3 c.c. each at the same time, and so on until the sixth-day cord is reached on the fifth day; two injections of the sixth-day cord emulsion are given in doses of 2 c.c. each at the same time; subsequent injections are: sixth day (fifth-day cord), 2 c.c.; seventh day (fourth-day cord),

2 c.c.; eighth day (third-day cord), $1\frac{1}{2}$ c.c. Injections are not given of cords earlier than the third day. Now begin again with fifth-day cord and come down to third-day cord inclusive; these all now being 2 c.c. doses.

"If it be thought desirable to approach at first the more virulent cords gradually, when the fifth-day cord is reached a fifth-day cord may be given again as the next day's injection; so also with the fourth-day cord, but after this reduplication the course of the injections is resumed and maintained in daily succession, fifth-day cord, fourth-day cord, third-day cord, and over again until the twenty-first day has passed, the dose being 2 c.c. each time." (Keirle.)

Regarding the *modus operandi* of the antirabic vaccination our knowledge is as yet imperfect, but it appears that as a result of the immunizing process *rabicidal substances* are formed which are capable of destroying the rabic virus. Babes accordingly combines the active immunization with the passive process, *i. e.*, the introduction of the serum of immunized animals, and apparently with satisfactory results. As rabicidal serum has no marked antitoxic properties, and as the symptoms of rabies are evidently toxic in origin, it is clear that no special benefits can be expected from its use when once the disease has developed.

Results.—So far as the results of the antirabic treatment are concerned an analysis of 31,330 cases, in which the existence of rabies in the biting animal had either been definitely established or rendered highly probable, shows an average mortality of but 0.75 per cent., which, no doubt, could be still further reduced if the treatment of the bitten persons could always be instituted in time.

After the disease has once developed, vaccine treatment is, of course, without avail; at present we can only hope that the future may yet teach us some method by which the disease when already in actual progress may yet be conquered and those unfortunates be saved from their terrible sufferings.

TYPHOID FEVER

After Pasteur had shown that it is possible to produce active immunity in animals against such diseases as chicken cholera, anthrax, and swine plague, the thought naturally suggested itself that the same should be possible in the case of some of the organisms which are pathogenic for man. Attempts in this direction showed, as a matter of fact, that it is perfectly feasible to protect the common laboratory animals against infections like typhoid and cholera, and that this end can be reached not only by the use of living cultures, but even with the killed organisms. The latter discovery is, of course, of the greatest importance, as it unquestionably hastened the application of the findings in the animal experiment to the prophylactic treatment of the human being. The great question naturally has been how large a dose of bacilli should be injected and how frequently the injections should be made in order to secure adequate protection. Pfeiffer and Kolle, who were probably the first to attempt this in the human being, thought that the bacteriolytic content of the serum might possibly be used as an indicator in this respect, while Wright, to whom we are indebted for the actual introduction of the method into common use, once thought that the opsonic content of the blood might prove of service in this respect. Subsequent studies, however, have shown that a parallel between the size of the dose, the serum content of protective substances, and the degree of immunity does not exist, and we may say that our present methods are essentially the outcome of actual trial, irrespective of any special index.

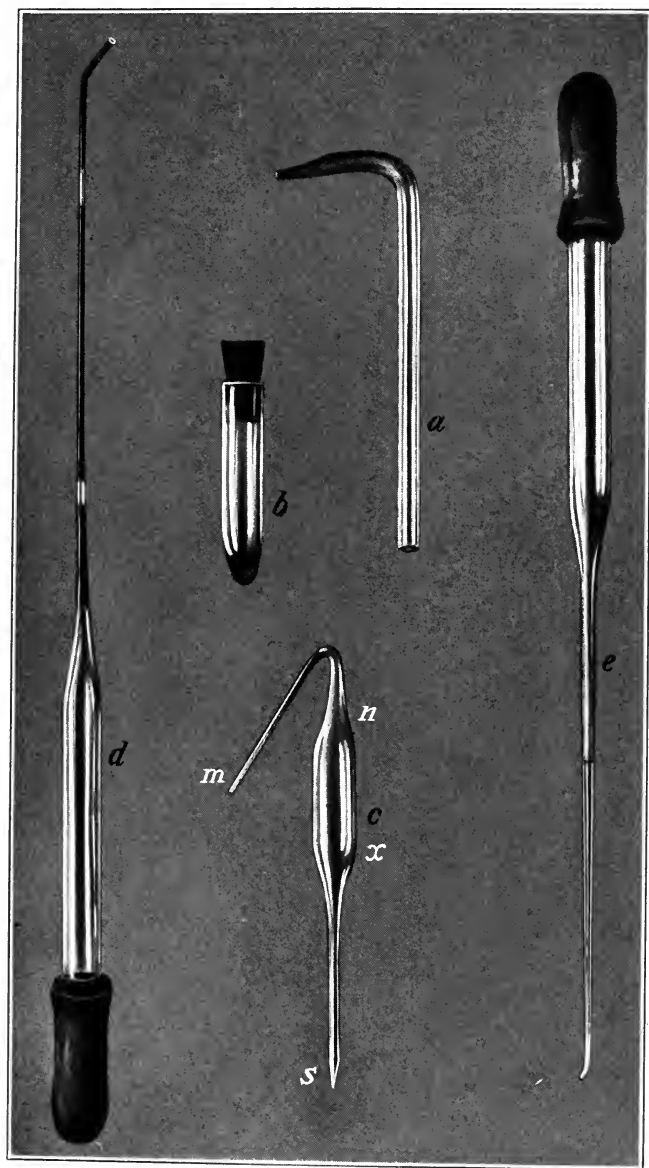
Preparation of the Vaccine.—Wright recommends that the culture from which the vaccine is to be made should first be brought to a certain degree of virulence by animal passage, and that its rate of growth in twenty-four hours should yield from 1000 to 2000 million bacilli per c.c. of bouillon. The first point can be reached by passing the organism through a few guinea-pigs, while the second has to be tried out. Whether either condition is really imperative may be questioned. To my mind it is more important that the vaccine should be polyvalent, *i. e.*, that it should represent a mixture of a number of different strains. As medium for growth ordinary 1 per cent. peptone broth is used, which should be as nearly neutral

as possible. Equal quantities of this, in suitable flasks, are inoculated and kept at body temperature for twenty-four to forty-eight hours, after which they are mixed, care being taken that the neck and upper portion of the mixing flask is not soiled by the emulsion, as otherwise some of the organisms may escape destruction when the mixture is sterilized, which is the next step in the operation. This is carried out in a suitable water-bath, at as low a temperature as possible; 60° C. is the usual temperature for this purpose, though some writers advocate 52° C. Care should be taken that the water in the bath stands at least as high as the culture in the flask, and that the temperature corresponds to the contents of the flask and not to the surrounding water. To this end a second flask, filled with water, is placed alongside the culture flask and a thermometer suspended in it. As soon as the temperature reaches 60° C., the flame of the water-bath is turned off. The flask remains in the hot water for ten to fifteen minutes longer, and is then removed. After this the sterility of the contents is tested by plating out a given quantity (1 to 10 c.c., according to the amount of material) in agar or by inoculation of broth. The content of bacteria is next determined as follows:

Determination of the Number of Bacteria (according to Wright).—A capillary pipette provided with a rubber nipple (Plate III, Fig. *d*) is marked with a glass pencil about three-quarters of an inch from the end, and is then charged with one volume of blood (obtained by puncture of the thumb near the root of the nail), one of the bacterial emulsion and three volumes of saline (0.9 per cent.), the individual portions being separated from one another by little air-bubbles.¹ Blood and bacteria are thoroughly mixed by repeatedly blowing the contents of the capillary upon a slide and drawing them up again in solid column. Small drops are mounted on clean slides, spread out like blood specimens, and, after drying, stained with Jenner's stain or one of the numerous Romanowsky modifications. A small square diaphragm made of paper or card-board is placed in the ocular of the microscope, when the red cells and bacteria are counted in successive fields until 1000 of the former have been gone

¹ With other bacteria, where the content may be much smaller one may take two or three volumes of the emulsion, instead of saline, due allowance being made in the calculation by dividing with two, three, or four, as the case may be.

PLATE III



Apparatus for Opsonic Work.

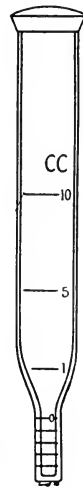
(a) pipette for collecting blood; (b) tube to receive blood for separation of serum; (c) Wright blood capsule; (d) blood pipette charged with corpuscles, serum, and bacterial emulsion; (e) same in solid column, ready for incubation.

over. As the number of red cells in 1 c.c. of normal blood is about 5,000,000,000, and as the red cells and bacteria must be present in the mixture in the same ratio to one another as in the original units of volume, the number of bacteria per cubic centimeter of the vaccine is ascertained according to the equation: Number of red cells counted : number of bacteria counted : : 5,000,000,000 : x . This method, of course, has no claims to accuracy, but it is the one which is usually employed in titrating out vaccines. A more accurate method has of late been suggested by Hopkins, which seems to have many points in its favor.

*Hopkins' Method.*¹—This is based upon the concentration of a bacterial culture by centrifugation and the preparation of standard emulsions from the sediment. To this end the washings from slant cultures, after filtration through a small cotton filter to remove larger clumps of bacteria and particles of agar, are placed in especially constructed centrifugation tubes (prepared by the International Instrument Company of Cambridge, Mass., see Fig. 15), covered with rubber caps, and centrifugalized for half an hour at a speed of approximately 2800 revolutions per minute. The salt solution and bacteria above the 0.05 mark are then removed and 5 c.c. of saline solution measured into the tube, so as to make a 1 per cent. emulsion. If the sediment does not reach the 0.05 mark its volume is read on the scale and a corresponding quantity of saline added to make the emulsion 1 per cent. in strength. By means of a capillary pipette armed with a nipple the organisms are forced into suspension, when the vaccine is transferred to a tube, to be killed in the usual manner.

Estimations of carefully counted suspensions obtained by centrifugation in the above manner gave the following results:

FIG. 15



Special centrifuge tube with graduated tip.

¹ Jour. Amer. Med. Assoc., vol. xl, No. 21, p. 1615.

	Per cent.		Billion per c.c.
Staphylococcus aureus and albus	1	=	10.0
Streptococcus hemolyticus	1	=	8.0
Gonococcus	1	=	8.0
Pneumococcus	1	=	2.5
Bacillus typhosus	1	=	8.0
Bacillus coli	1	=	4.0

With different speeds different standards will, of course, be obtained.

After the strength of the emulsion has been determined the material is diluted to the desired degree with carbolic acid, such that the final content of the latter shall be between 0.25 and 0.5 per cent. This then constitutes the vaccine and keeps practically indefinitely. The manufacturers now furnish this in little ampoules containing the requisite dose.

Dose and Method of Vaccination.—For practical purposes Wright recommends a first injection of 750,000,000 to 1,000,000,000 organisms, and double this dose for the second treatment. These injections may be made practically anywhere, where the skin is not bound down tightly. I have thus found the outer aspect of the upper arm, where the skin lies quite loose, a favorable locality. Others inject in the loin, or in the back, in the neighborhood of the shoulder-blade. The injections are, of course, to be made with a sterile syringe, and after having cleansed the area to be injected with soap and water and alcohol, or, as has recently been recommended, after painting with tincture of iodine. The needle puncture is covered with collodion.

Fearing that the injection of a large dose of organisms may at first be followed by a diminution in the protective substances of the body (negative phase), owing to an interaction between the normal antibacterial substances and the bacterial antigen, and that the individual may thus be temporarily less resistant to the corresponding infection, Wright has suggested that in persons who are likely to be exposed to typhoid infection soon after the first injection, this should be smaller than usual, and that its effect is to be supplemented later by a correspondingly stronger injection. Whether or not such a danger actually exists in the case of the human being has not yet been demonstrated. In the animal experiments, such a period of diminished resistance apparently does not develop, for Pfeiffer and Friedberger have shown that guinea-pigs which have been vaccinated

with large doses manifest an increased resistance as early as eight to thirty-six hours following the injection.

Symptoms following Vaccination.—Usually within two or three hours following vaccination a local reaction appears about the site of inoculation, which is characterized by marked redness and infiltration. This is followed by elevation of temperature (up to 102° F.), and occasionally by headache, general lassitude, muscle pain, and frequently by swelling of the regional lymph glands. These symptoms persist usually for one to three days and then disappear. Individually there is a good deal of variation, both in the extent of the local reaction and in the intensity of the general symptoms. The majority of people are very little inconvenienced, and are able to continue about their work as usual. In isolated cases, however, the person may feel quite ill for a number of days, and suffer a good deal of pain in the arm, which may be red and swollen over a considerable area. Prompt recovery takes place in every instance. For the local pain, Wright recommends warm applications and a salve composed of carbolic acid, 1.0; fld. ext. of ergot, 4.0; oxide of zinc, 3.0; lanolin, 20.0; for internal use he recommends 2.0 grams of calcium chloride or calcium lactate. All observers remark that in malarial cases the reaction is unusually severe, and had better be avoided. The symptoms occurring after the second injection are usually milder; Leishman noted marked sweating during the second night following the reinjection. During the twenty-four hours following the inoculation the individuals should be told to abstain from the use of alcohol, and to avoid muscular exertion and exposure to the sun.

Following the inoculations, there is a marked increase in the bactericidal substances and bacteriolysins of the blood, which reaches its highest point on the third day following the reinoculation (fourteenth day) in the case of the first, and on the seventh day (eighteenth day) in the case of the latter. Generally speaking the increase amounts to from five to ten times the original quantity. Agglutinin formation begins on the ninth day, drops after the second injection, and starts again nine days later, reaching its maximum between the twenty-second and twenty-fifth day (2000 to 4000). Normal opsonins, according to Leishman, are not demonstrable, while the stimulins (immune opsonins, *i. e.*, elements occurring in heated serum, which favor phagocytosis) are increased after eleven days.

The Duration of the Protection.—The duration of the protection afforded by the vaccination Wright estimates at from two to three years, while Kuhn speaks of a single year.

Results.—In the human being it is, of course, out of the question to study the protective value of the vaccination, as is possible in the animal experiment. All that we can do is to compare the rate of morbidity from typhoid fever in a large body of vaccinated individuals who have been more or less exposed to infection, with that occurring in a similar body of men who have not been protected, and who have been exposed to a similar extent. We can further compare the rate of mortality among the non-vaccinated with that of those who have been vaccinated, but who have nevertheless developed the disease. Studies of this kind have been carried on in the English army at the time of the Boer War, in the German army in Southwest Africa, and lately in the United States concentration camp on the Mexican border (1911).

Some of the data obtained in the English army are given in the accompanying table, from which it is clear that the vaccinated individual enjoyed a much greater security, both as regards the probability of infection, and the outcome, in the event that the disease nevertheless developed:

	Men.	Non-vaccinated.		Men.	Vaccinated.	
		Morbidity.	Mortality.		Morbidity.	Mortality.
Indian Army (1899)	25,851	657 (2.54%)	146 (0.56%)	4502	44 (0.89%)	9 (0.2 %)
Garrison of Ladysmith (1899 to 1900)	10,529	1489 (14.14%)	329 (3.12%)	1705	35 (2.05%)	8 (0.47%)
Army in Egypt and Cyprus	2,669	68 (2.55%)	10 (0.37%)	720	1 (0.14%)	1 (0.14%)
Hospital at Bloemfontain	178	24 (14%)	53	3 (5.6 %)

From this table it is also clear that the protection is not absolute.

The results obtained in our own army are even more striking. It will be recalled that both the morbidity and mortality from typhoid fever in our concentration camps at the time of the Spanish war were perfectly appalling. In a body of 10,759 men there were thus 1729 cases of certain typhoid, and in addition 964 cases of

probable typhoid (2693 in all), with 248 deaths. Translated into percentages this means that of the entire body of soldiers 25 per cent. were taken ill with fever, which either was definitely recognized or suspected as being typhoid, with a death-rate of 9.2 per cent. All these men were non-vaccinated. Compare with this the fact that among the 12,801 men who were concentrated in 1911 at the maneuver camp at San Antonio, all of whom had been vaccinated either before their arrival or as soon thereafter as possible, there developed but a single very mild case, a private who had not completed his immunization, thus giving a percentage of 0.008! During the same period there were reported in the city of San Antonio forty-nine cases and nineteen deaths.

On the basis of these findings it would appear that with modern sanitary methods, coupled with vaccination in the case of those who are likely to be exposed to infection, typhoid fever should ere long become as rare in our hospitals as are smallpox and cholera at the present time.

Antityphoid Vaccination for Curative Purposes.—Since the introduction of antityphoid vaccination for prophylactic purposes various attempts have been made to influence the *course of the malady* also by such measures. Some writers indeed express themselves quite favorably on this point, but it will no doubt require a great deal of investigation before we can come to any definite conclusions. It should be remembered that we have no indicator to tell us how much to inject and when to inject, and aside from the actual course of the malady, which varies so greatly in different cases, we have no way of knowing whether we are producing an effect at all, let alone whether this is beneficial or otherwise. Whether or not Wright's opsonic index might yet serve some purpose in the study of such cases the future will have to show.

CHOLERA

Prophylactic vaccination against Asiatic cholera was first attempted in 1885 by Ferron, during an epidemic occurring in Spain. As infection of the human being with the organism in question can only take place by way of the intestinal canal, Ferron injected *living* organisms subcutaneously, using eight drops of a broth culture for

the first, and 0.5 c.c. for the second and third, the inoculations being made six to eight days apart. His statistics and experiments on guinea-pigs, which formed the basis of his work on the human being, have been adversely criticised by a number of subsequent investigators; but the fact remains that he was the first to attempt anti-cholera vaccination in the human being, that he injected a large number of people (200,000, according to his statements) with living cultures, and that his method is essentially the same as that which Haffkine subsequently used in India, and which unquestionably can afford protection. We also know that as a consequence of such injections bactericidal substances appear in the serum in large amounts, and that attempts in this direction hence have a proper theoretical basis.

The essential difference between Ferron and Haffkine is that the latter uses an attenuated culture (vaccine I) for his first injection, and then follows this with one that has been brought to a high degree of virulence by animal passage, and which in conformity with Pasteur's nomenclature, he speaks of as *virus fixe* (vaccine II). Later investigations have shown, as a matter of fact, that a high degree of virulence is essential to effect successful immunization. But, like Ferron, Haffkine thought it imperative to use living cultures. That this is unnecessary, however, was subsequently shown by Kolle, and the results which have thus far been obtained with the latter's method, both in the human being and in the animal experiment, seem to render future work with living cultures unnecessary and perhaps even undesirable.

Kolle's Method.—The vaccine is prepared by emulsifying twenty-four-hour-old cultures of a virulent strain (increased by passage through guinea-pigs) of the cholera vibrio in normal salt solution, such that 1. c.c. shall contain 1 oese (= 2 mgrms.) of organisms. These are then killed by exposure to 60° C. for one-half hour, when carbolic acid is added, to the extent of 0.5 per cent., as preservative. Two injections are given hypodermically about a week apart, 1 c.c. the first time and 2 c.c. the second time. Care should be taken not to inject at a place where the skin is bound tightly down. Suitable districts are the area over the triceps and the loin.

Symptoms following the Inoculation.—As in the case of the anti-typhoid injections, the symptoms vary in different people. Locally there is more or less pain which begins after five to six hours, with

relatively little redness and swelling. There is usually some elevation of temperature (101° to 102° F.), headache, and general malaise; in women nausea and vomiting, and in about 10 per cent. of the people diarrhea on the following day. After twenty-four to seventy-two hours the symptoms have disappeared.

Results.—Kolle's method has been tested in Japan (1902), and has apparently furnished reasonably satisfactory results, even though the vaccination, as in the case of the antityphoid treatment, does not afford protection in all cases. In a certain district occupied by 903,194 people, Murata vaccinated 77,907 individuals, the result being that the morbidity among the latter was only 0.06 per cent., as contrasted with 0.13 per cent., when compared with the total population, and the mortality (calculated in relation to the morbidity) only 42.5 per cent., as compared with 75 per cent. In actual figures this means that of 825,287 non-vaccinated people 1152 people contracted the disease, resulting in 863 deaths, while of 77,907 vaccinated individuals only 48 were taken ill and 20 died.

Even more convincing than these figures are certain individual observations. In two villages which were close to a large cholera focus, and in which all the inhabitants had been vaccinated, not a single individual was taken ill, notwithstanding a most active intercourse between the people.

In a branch office of the Formosa Camphor Company all but three individuals were inoculated (159). But one of the total number, and this one a non-vaccinated person, developed the disease and died.

Similar results have been obtained by Haffkine in India, so that the conclusion seems justifiable that vaccination with suitable material actually affords a considerable degree of protection against Asiatic cholera, and should be enforced as far as possible in times of epidemic. Coupled with modern sanitary methods, vaccination should certainly remove a great deal of the danger which attaches to this relic of medieval lack of civilization.

PLAGUE

Attempts at prophylactic vaccination against plague have likewise led to encouraging results. Haffkine, who has done a great deal of

the pioneer work in this direction, thus gives some very convincing figures: In the city of Hubli (British India), numbering about 47,427 inhabitants, vaccination was begun on the 11th of May, 1898. From this date until the 27th of September 38,712 individuals had been vaccinated, and of these 339, *i. e.*, 0.8 per cent., died. Of the non-vaccinated during the same period 2395 succumbed, *i. e.*, 5 per cent., as compared with the total number of inhabitants. During the week of September 21 to September 27, when all the inhabitants, with the exception of 603, had finally been vaccinated, there were among the 38,712 protected individuals only 20 deaths, while of the 603 non-vaccinated persons 58, *i. e.*, 9.61 per cent., died.

Quite striking also are the following data: In three villages there occurred 13 cases among 365 vaccinated persons, with 3 deaths, while of the 363 non-inoculated individuals 49 were taken ill and 38 died. In Bombay there developed 18 cases of the disease among 8200 vaccinated persons, with 2 deaths (mortality 11.1 per cent.), while the general mortality from the disease was over 90 per cent.

These few examples will, I think, suffice to illustrate the real value of vaccination against plague, but it will be noted, as in anticholera vaccination, that the protection is not absolute. The mortality among the vaccinated is so much lower, however, *i. e.*, 11 to 41 per cent., as compared with 50 to 92 per cent., among the non-vaccinated, as observed in different localities, that this factor in itself would establish the value of the procedure, and as a matter of fact all the different commissions, which have investigated the Haffkine method, have expressed themselves in this sense.

The Duration of Protection.—This is estimated at several months, after which the vaccination must be repeated, if danger of infection still exists.

Preparation of the Vaccine. (Haffkine).—Haffkine makes use of bouillon cultures which have been allowed to grow for six weeks at a temperature of 25 to 30° C. The bouillon is prepared as follows: 1000 grams of lean (goat) meat are passed through a meat-hashing machine, and are digested for three hours with 125 grams of hydrochloric acid (concent.) in the autoclave, at a pressure of three atmospheres. The resultant material is filtered and diluted with water, so that the content in proteins shall be 1 per cent. (approximately seven volumes of water). The broth is then neutralized with

calcium carbonate, and sodium chloride added to make it of physiological strength. It is then sterilized and filtered into suitable flasks (to a height of 7.5 cm.), into each of which a few drops of olive oil or butter fat (sterile) are further added, to serve as floats from which surface growth of the bacilli can take place. Every two or three days the cultures should be shaken, so that new crops of the organism can develop in contact with the air, the older ones going to the bottom.

After a six weeks' growth has been obtained, the purity of the culture is examined by plating out a small amount on agar. The material is sterilized for one or more hours at 65° C., treated with carbolic acid to the point of 0.5 per cent., and finally filled into small vials of 30 c.c. capacity.

Dose.—The ordinary dose for adult males is 3 to 3.5 c.c.; for adult females, 2 to 2.5 c.c.; for children more than ten years old, 1 c.c., and for smaller children, 0.1 to 0.5 c.c. Much larger quantities, however, can be employed without harm, and Haffkine himself has injected as much as 20 c.c. A second injection is recommended after eight to ten days. The injections are made subcutaneously, with a sterile syringe, into the upper arm (area over the triceps) or the loin, those districts being avoided, as usual, in which the skin is tightly bound down.

Symptoms following Injection.—The symptoms following the injection are practically the same as those occurring after an anti-cholera or antityphoid vaccination, and differ considerably in their severity in different people. After twenty-four to forty-eight hours the individuals are usually no longer inconvenienced.

After the disease has once developed vaccination is of no avail, and in such cases serum treatment should be resorted to (see below). The *combined procedure* would suggest itself as being of value when there is reason to think that the person may have already been exposed to the infection or when great danger actually exists. For such purposes Shiga advocates an initial treatment of 0.6 to 1 c.c. each, of antiplague serum and vaccine, which is to be followed after a few days, *i. e.*, after the reaction has disappeared, by a second injection of vaccine alone. The latter is essentially an emulsion of three-day-old agar cultures (incubated at 30° C.), 1 c.c. of physiological salt solution being used for each oese of the culture. The emulsion is kept for 30 minutes at 60° C., treated with carbolic acid

to the extent of 0.5 per cent., and allowed to stand for twenty-four hours before being used.

During epidemics Shiga recommends that still larger doses of the vaccine be used, or to vaccinate three times with increasing quantities.

DYSENTERY

Protective vaccination against bacillary dysentery has likewise been attempted, but has not as yet led to results which are comparable in value to what we see in the case of plague and cholera. Shiga himself inoculated some 10,000 individuals between 1898 and 1900, and thought that he could note a decrease in the mortality, while the morbidity and the severity of the symptoms were apparently uninfluenced. The dead cultures, however, are so highly toxic that this element in itself is an obstacle to the more general use of such a vaccine. Whether further investigations in this direction will lead to more practical results time only can tell, but it would not seem to be out of the question, particularly when coupled with the use of a corresponding antitoxic serum.

OTHER DISEASES

In the other infectious maladies to which the human being is subject protective vaccination has either not yet been attempted or has not yielded encouraging results. There are a number of infectious diseases, however, occurring in the domesticated animals against which vaccination may be successfully employed. This is notably the case with anthrax, swine plague (Schweinerotlauf), cattle plague (Rinderpest), sympathetic anthrax (Rauschbrand), and within certain limitations also with cattle tuberculosis. For a consideration of the methods employed and the results which have been reached in these diseases which so closely affect the human being the reader is referred to special works.

(B) ACTIVE IMMUNIZATION FOR THERAPEUTIC PURPOSES

While in the diseases which have thus far been considered, active immunization will only furnish results of value when carried out for *prophylactic* purposes, whereas the same measures have no influence,

so far as we know, upon the disease, when this has once been established, it appears from recent studies that vaccination may advantageously be employed as a *curative* agent, in those infections which are characterized by a tendency to chronicity, and in which toxins play little or no role. The credit for having established this possibility, and for its popularization, undoubtedly belongs to Wright.

Wright's concept of the rationale underlying the tedious course of some of these infections is essentially based upon the supposition that the autovaccinations which take place in the body of the infected individual are imperfectly interspaced as regards point of time and improperly adjusted as regards dosage, the consequence being that the formation of certain protective substances, and notably of the opsonins, takes place irregularly and insufficiently. He expressed the opinion that by following the opsonic curve indications might be obtained for the introduction of the corresponding organisms from without as vaccines, both as regards the size of the dose and the frequency of the injections, and that it might thus be possible to favorably influence such infections as acne, sycosis, furunculosis, endocarditis, chronic cystitis, pyelitis, tuberculosis, etc. For a consideration of the details underlying Wright's opsonic studies, I must refer the reader to Wright's own publications, and the chapter on the opsonins in the first part of the present work. Suffice it to state at this place that the opsonic index unfortunately did not fulfil those expectations with which it was at first greeted, and that any attempts at vaccine treatment must still be made upon a more or less empirical basis, and with no more definite or accurate index to dosage and frequency of injection than is afforded by the clinical symptoms. But even so, there can be no doubt that a certain amount of good may be accomplished; how much, it is yet impossible to say. So much depends upon the individual case, coincidence, the personal factor in the observer, etc., that conclusions should only be drawn with great care. As yet we certainly do not know enough of what may or what may not be accomplished to warrant any dogmatic statements.

Preparation of the Vaccines.—A great deal of discussion has arisen regarding the question whether or not it is imperative to use *autogenous vaccines*, *i. e.*, vaccines that are derived from the individual organism which is responsible for the particular infection, or whether it is permissible to make use of *stock vaccines*, which may in turn be

polyvalent, *i. e.*, composed of organisms derived from a number of cases of the kind that is under consideration, or from one single case, but not from the individual who is to be treated. As long as we know so little of what vaccines may accomplish it is clear that our clinical knowledge is not sufficient to decide such a question. We can only speak theoretically, and theoretically we must admit the probable existence of many strains of a given type of organism, and with this the possibility of individual differences, so that upon this basis autogenous vaccines would, *cæteris paribus*, appear to be preferable to stock vaccines. But as it is frequently and in some infections indeed uniformly impossible to prepare an autogenous vaccine, we may be forced to use stock material in many cases.

The preparation of the majority of vaccines is conducted as follows: Cultures are made from whatever source is available, *i. e.*, from the pus of abscesses, from acne pustules, the urine, blood, sputum, etc., the culture medium being chosen in accordance with the type of organism that is expected, or which a preliminary examination has shown to be present. If only one type is found, the vaccine is made from it, while in the event of a multiple infection, or in the presence of contaminating saprophytic organisms, the predominating pathogenic varieties are chosen, *i. e.*, those which are recognized as being pathogenic either by direct examination or by passage through an animal.¹

Having once secured an initial supply, it is then only necessary to inoculate a sufficient number of tubes or flasks of agar, or serum agar, and to incubate these as usual. With organisms that furnish a prolific growth, incubation for twenty-four hours is sufficient, while with the more delicate organisms, such as the streptococcus and pneumococcus, it is advisable to wait for forty-eight to seventy-two hours. At the expiration of this time a small amount of sterile saline solution (10 c.c. to an ordinary agar slant) is poured into the first tube and the growth gently scraped off with a platinum loop. The emulsion is then poured into the next, from this into the following, and so on, according to the number of tubes or the quantity of vaccine which is to be prepared.

¹ In this connection I cannot condemn too strongly the use of some of the so-called polyvalent and mixed vaccines which have been recently placed upon the market with the most extravagant claims for their efficacy in the absence of any proof of their value.

The general idea is to make an emulsion at the start that is stronger than the one desired in the end, and subsequently to dilute this to the required degree. The emulsion is transferred from the last tube to a sterile test-tube, care being taken that the fluid does not come in contact with the neck of the tube, as otherwise some organisms may dry here and subsequently escape sterilization. This is then carried out in a water-bath at a temperature of from 60° to 65° C. An exposure of one hour is sufficient, counting from the time that the contents of the tube reach the desired point. A number of sterile glass beads are now added, and the tube, tightly closed with a sterile stopper, shaken by hand or with a machine for about fifteen to twenty minutes. The bacterial content is then ascertained, as described above (see Preparation of Typhoid Vaccine, p. 190). As diluent I use an 0.5 per cent. solution of carbolic acid, taking care that the final content of the latter, in the finished vaccine, does not fall below 0.25 per cent. The tube is then allowed to stand on end overnight (so as to test the sterility of that portion of the tube), and a culture made the next day, using a good-sized drop for each tube, which is conveniently placed in broth or milk. The preparation is finally provided with a label, giving the name of the organism, and the titer of the vaccine per 1 c.c. If desired, the vaccine can, of course, also be put up in glass beads or ampules, each containing a single dose of 1 c.c. In this form the material is usually furnished by the dealers.

While the common bacterial vaccines may be prepared in the clinical laboratory from autogenous material, this is out of the question in the case of the tubercle bacillus. Such a vaccine is best obtained from the dealers, and is sold under the name of Koch's *Neu (new) Tuberculin*, bacillary emulsion). It is prepared by carefully grinding fresh cultures of the bacillus, after being dried in the vacuum, in an agate mortar, or in a specially constructed mill, when the organisms are emulsified in equal parts of water and 50 per cent. glycerin (100 parts of each for one part of bacilli). 1 c.c. of this preparation contains 5 mgrms. of bacilli, and from it the required dilutions are made, care being taken to sterilize the stock solution before diluting, by exposure to a temperature of 60° C. for one hour. As diluent a 0.25 per cent. solution of lysol in physiological salt solution is used.

The Injection.—If the vaccine has been put up in bulk a small quantity is poured into a small medicine glass that has just been boiled, and the (sterilized) syringe charged from this; or this is filled from one of the ampules directly. The skin is scrubbed with soap and water and then with alcohol, or, as is now recommended, painted with tincture of iodine at the site of the injection. My favorite site for this is the district over the triceps, into the loose subcutaneous tissue. In this region the injections rarely give rise to painful local reactions, while injections at a point where the skin is tightly bound down is almost sure to cause a good deal of avoidable distress. For this reason also intradermal injections are to be avoided.

Dosage and Frequency of Injection.—As I have already indicated, we have as yet no satisfactory index to dosage. As the first principle of therapeutics is *noli nocere*, it is advisable to begin with small doses, *i. e.*, with quantities which past experience has shown to do no harm, so far at least as we can judge this by clinical evidence.

At the present time the injections are usually given about a week apart, the size of the dose being increased at each sitting. If no change results from the treatment, larger doses may be tried; and I may say that we have sufficient evidence to show that much larger doses than the maximal quantities now recommended *may* be given in most cases. In one or two instances I have indeed received the impression that the patient owed his recovery from serious illness to the injection of a quantity of organisms, which was many multiples of the maximal dose usually recommended. If the symptoms become aggravated the dose should be diminished and the ascent carried out less abruptly and possibly at somewhat longer intervals (ten to fourteen days or longer). Generally speaking, in the more acute cases, the smaller doses should be chosen, to begin with, and the larger ones reserved for the more chronic ones. There are, however, no hard-and-fast rules to be laid down at the present time. The would-be immunisator must learn from experience, and should not pay too much attention to “negative and positive phases” when these are based on the feelings of well-being, and of depression on the part of the patient. He should be neither of too optimistic nor of too pessimistic a temperament, and should weigh the evidence with a calm and unbiased mind; in other words, he must be an exceptional individual. I really know of no field in medicine at the present day

where it is possible to draw so many erroneous conclusions regarding the value of a therapeutic agent as in the domain of vaccinotherapy in its application to chronic infections. Much good can unquestionably be accomplished, but we must be careful not to attribute all improvement to our immunizing efforts.

Standard Doses.—As standard doses of the different vaccines, or *bacterins*, as bacterial vaccines are now termed, the following may be recommended, bearing in mind what has been said in the foregoing lines:

Staphylococcus aureus	50,000,000 to	500,000,000 (or more)
Staphylococcus albus and citreus	100,000,000 to	1,000,000,000 (or more)
Streptococcus pyogenes	5,000,000 to	100,000,000 (or more)
Gonococcus	5,000,000 to	100,000,000 (or more)
Friedländer's bacillus	10,000,000 to	100,000,000 (or more)
Colon bacillus	10,000,000 to	100,000,000 (or more)

Of the tubercle vaccine it is recommended to begin with very small doses, *i. e.*, $\frac{1}{30000}$ to $\frac{1}{15000}$ of a milligram, and to continue the same dose or gradually increase it, according to the indications of the individual case (see Tuberculosis).

Indications for the Use of Bacterial Vaccines.—As I have already indicated in a general way, bacterial vaccines may be employed in practically any infection which has a tendency to a certain degree of chronicity. It has been recommended in the various chronic infections of the bones and joints (tuberculosis, osteomyelitis, gonorrheal arthritis) in the early stages of pulmonary tuberculosis, in chronic gonorrheal infections, in the colon bacillus infections of the urinary tract, in tubercular cystitis, in the chronic staphylococcus infections of the skin (lupus, scrofuloderma, tuberculides), in chronic inflammation of the middle ear, the antrum, the frontal sinus; also in endocarditis, as a postoperative measure in connection with empyema, etc.

While acute infections have generally been regarded as contra-indicating the use of vaccines, this has largely been on theoretical grounds. Personally, I have gained the impression that vaccination, even in such cases, could do some good. However, it is just in such cases that correct judgment is frequently fallacious and difficult.

To what extent vaccination may be serviceable as a protective measure before certain operations is difficult to say. Generally speaking, one should expect that it might be of use in those cases

in which there is danger of infection, and in which enough time is given before operations to attempt the patient's immunization, while in urgent operations the measure would seem to be contra-indicated as possibly favoring infection, *i. e.*, as causing an immediate, though temporary, drop in the body's content of protective substances.

Results.—It is evident from what has been said that we know too little as yet of what vaccination can accomplish in the chronic bacterial infections to warrant any dogmatic statements. The amount of clinical material that has been satisfactorily studied is entirely too small as yet, and it seems to me that we are really not entitled to say more than that vaccination *may* do good and should be tried. But we can neither state in what percentage of cases it will be helpful or effect a cure, nor can we predict in an individual case what the result will be. I have seen excellent results, and no results, in apparently similar cases, and I feel that every unbiased observer has similar experiences to record. It would after all be expecting a great deal, in the absence of any more delicate indicator to what is going on in the offensive-defensive interaction in the body, than coarse clinical symptoms, to be obliged to predict what will happen in a given case and whether we are doing the best that can be done.

While I have pointed out in the foregoing pages that we are not yet in a position where we can speak definitely of the results of vaccine treatment and its indications or contra-indications, I must modify this statement somewhat, so far as tuberculosis is concerned, and it may not be out of place to consider this special question by itself and in some detail.

VACCINE TREATMENT OF TUBERCULOSIS

The earliest attempts to influence the course of tuberculosis through active immunization were made by R. Koch, and were based upon the observation that a tubercular guinea-pig reacts quite differently to a subsequent inoculation with tubercle bacilli than does a normal animal. For whereas in the latter a tubercular ulcer develops at the site of the injection which does not heal, but persists until the animal dies, local recovery occurs in the tubercular guinea-pig without involvement of the regional lymph glands.

Evidently then the first inoculation, even though it leads to the death of the animal, produces a certain degree of resistance, which the untreated animal does not possess, and it very naturally occurred to Koch that it might be possible to utilize this observation as a basis for the treatment of human tuberculosis. Further indications for experiments in this direction were afforded by the finding, that, whereas the injection of large numbers of tubercle bacilli hastens the death of the tubercular animal, small doses, frequently repeated, seemingly have a beneficial influence both upon its general condition and upon the progress of the lesion at the site of the primary inoculation. Since killed-off cultures, however, though quite efficacious, were "apparently not resorbed from the point of inoculation, nor otherwise removed, but remained there undisturbed and produced abscesses," Koch attempted so to modify his vaccine as to separate the curative from the harmful principle. The result was his famous *tuberculin*. This was prepared by growing tubercle bacilli for eight weeks in 4 per cent. glycerin-peptone-bouillon, and then concentrating the culture at 90° C. to one-tenth of its original volume. The resultant material thus actually represents a 40 per cent. glycerin extract of the original culture, which is finally passed through a porcelain filter, and is then ready for use.

This is hardly the place to narrate the history of the introduction of Koch's tuberculin into clinical use, the hopeful anticipation with which it was received, and the sorrowful disappointment that was the outcome of its earlier clinical trials. Suffice it to recall that the medical profession for a while expected the impossible, and that the non-realization of these expectations caused the pendulum to swing the other way, and to such a degree in fact that even now the very word "tuberculin" to most minds suggests failure. This was largely the outcome of the indiscriminate use of the substance, and the fact that in most cases the final verdict was based upon a trial, scarcely extending over a longer period than a month or two even in cases where subsequent experience has shown that recovery under its use is possible. That this may indeed occur, and more promptly so than under an expectant plan of treatment, seems to be undeniable; but detailed clinical studies have shown that the successful immunization of the tubercular individual is frequently beset with great difficulties, owing to the existence or development of a curious and most remarkable hypersusceptibility, in consequence of

which every injection is followed by a reaction which is evidently detrimental to the patient.

In our account of anaphylaxis we have already drawn attention to this occurrence and have studied the mechanism which underlies its production. I would merely emphasize at this place that in tuberculosis, probably more than in any other of the infectious diseases, anaphylactic processes are responsible for many of the phenomena which go to make up the clinical picture of the disease. In the days when Koch's early work on tuberculin was done, nothing was as yet known of anaphylaxis, and the symptoms following its injection were generally attributed to associated toxic substances. Koch hence directed his efforts to the preparation of a less toxic product, especially as he had not succeeded in producing an actual immunity in his experimental animals with the old tuberculin.

As it was out of the question to use killed-off cultures as such, owing to the production of abscesses when used hypodermically, or the formation of nodules in the lungs when injected intravenously, Koch resorted to the following procedure: Young cultures which had been dried in the vacuum were ground to pieces in a specially constructed apparatus, and the resultant powder shaken with distilled water, and then centrifugalized. The residue constitutes Koch's so-called T. R. preparation, while the supernatant fluid was termed T. O. Subsequently he brought out his New (neu) Tuberculin, which is practically an aqueous emulsion of the entire organisms, pulverized to mere fragments, and preserved by the addition of 50 per cent. of glycerin.

Although the use of the old tuberculin is still continued, this new product is rapidly gaining in favor and virtually corresponds to the bacterial vaccines which we have considered heretofore. Its antigenic power is proved by the fact that on treatment with this material the agglutinin titer of the patient's serum is frequently raised as high as 1: 500. This, to be sure, does not constitute an index to the degree of immunity which is produced, but it proves that the substance in question has the power to bring about that general allergic state of which agglutinin production is one of the possible manifestations.

Dosage and Injection.—*Old Tuberculin.*—The old tuberculin is put up in 1 c.c. and 5 c.c. ampules. Unless a very large number of

people are to be injected at one time it is better to use the smaller size. From this four dilutions are prepared by starting with a 1 in 10 (A) of the original strength, by then making a 1 in 10 from this (B); a 1 in 10 from that (C), and a 1 in 10 from the last (D), using sterile water as diluent, and working with sterile glassware. As 1 c.c. of the original product represents 1000 milligrams of the pure tuberculin, 1 c.c. of dilution A will contain 100 milligrams, 1 c.c. of B 10 milligrams, 1 c.c. of C 1 milligram, and 1 c.c. of D 0.1 milligram; from which latter further dilutions can be prepared according to the same plan, as desired.

As initial dose, that amount is suggested which a preliminary diagnostic examination has shown to produce a systemic reaction (see Tuberculin Test). If this is for any reason omitted, it is best to start the patient with 0.1 milligram, and to increase the dose progressively at intervals which are determined according to the activity of the reaction, and which accordingly vary from one to two weeks. In the event of a marked reaction, it is best to repeat the last dose, or to increase this only very slightly. A reversion to a smaller dose is to be avoided, and it is better, if need be, to wait a week longer before the next injection is given. In the light cases it is thus possible to run up to a dose of 1000 milligrams without much trouble, while in the presence of more advanced lesions this is more difficult; when the higher doses are reached the intervals between the injections may have to be lengthened to a month or even longer. The treatment is virtually considered at an end when the patient can stand a dose of 500 milligrams without marked systemic reaction.

New Tuberculin.—1 c.c. of new tuberculin represents 5 milligrams of the dry powder. Koch recommends that the injections be started with a dose of 0.0025 milligram. To this end the original product is diluted with sterile water to the required degree, so that the amount to be injected is less than 1 c.c. in bulk, as larger quantities favor the development of local infiltration. For convenience' sake we can start with a dilution of the original product of 1 in 10 (A), 1 c.c. of which in turn is diluted 1:10 (B), and of this again 1 c.c. in the same proportion (C); 1 c.c. of A then contains 0.5 milligram, 1 c.c. of B 0.05, and 1 c.c. of C 0.005; 0.5 c.c. of the latter dilution being thus the initial dose.

The injections are at first given four days a part, and later when

reactions begin to appear (*i. e.*, when amounts varying between a tenth and one-hundredth part of a milligram are injected) at intervals of eight days, in gradually increasing amounts, such as $\frac{1}{400}$ milligram; $\frac{3}{400}$, $\frac{5}{400}$, $\frac{2}{100}$, $\frac{3}{100}$, $\frac{4}{100}$, $\frac{5}{100}$, $\frac{6}{100}$, $\frac{1}{10}$, $\frac{2}{10}$, $\frac{3}{10}$, etc., exactly as was customary with the old tuberculin. Koch advocates that the immunization be continued until the patients can take 20 milligrams of the dry powder without any reaction.

Point of Injection.—As in the case of the other bacterial vaccines the *injections* can be conveniently given into the loose subcutaneous tissue in the district over the triceps, or in the back on a level with the angle of the scapula. Löwenstein states that the injections may be advantageously given intravenously, that the infiltration of the skin is thus obviated, and that the reactions are of briefer duration.

In advanced cases of pulmonary tuberculosis a combined treatment with old and later with new tuberculin has been recommended. In this connection, Bandelier advises that when the change from the old to the new is made, to begin with the two-hundredth part of that dose of the old tuberculin which produced no reaction.

While Koch emphasizes the importance of steadily increasing the dose, Wright does so only in the beginning; later on he continues with a constant dose. His initial quantity is much smaller than that recommended by Koch, viz., 1 c.c. of a dilution of the new tuberculin of 1:200000. Each dose is repeated a week or ten days apart, and then increased by one-fifth to one-sixth, until 1 c.c. of a dilution of 1:50000 to 1:10000 is reached, after which the final dose is continued (without further increase) for a number of months.

Time of Injection.—As soon as reactions begin to appear, it is advisable to give the injections in the morning, so that the patient is not disturbed by the febrile movement during the night. If this is at all marked, Koch recommends that the injections be stopped, and resumed two or three days after the temperature returns to normal, the same dose being given as the one preceding.

Still another procedure has been recommended by Wolff-Eisner, which was planned with the idea of developing receptors for the tubercular poison in the cutaneous connective tissue, *i. e.*, in vitally unimportant structures, in which the poisons in question that are formed in the diseased organs can be anchored. The method is the

following: The existence of a cutaneous susceptibility to the action of tuberculin is first established by intracutaneous injection of tuberculin in different concentration ($\frac{1}{1000}$ milligram, or, if this give a negative result, of $\frac{1}{100}$ or more). One-third of a c.c. of a solution containing $\frac{1}{1000}$ or $\frac{1}{100}$ milligram to the c.c., as the case may be, is then injected intracutaneously at each one of two or three different points, a platinum-iridium needle being conveniently used for the purpose. These injections are repeated at intervals of from four to eight days, the same dose being used as long as this is followed by a local reaction. The maximal dose is rarely more than one-tenth of a milligram.

Indications and Contra-indications.—Anyone who has seen some of the disastrous results which followed the use of tuberculin in the early days of its history, will realize that not all cases of tuberculosis are suitable for the tuberculin treatment. Now we know that it is best to exclude those cases in which there is any febrile movement of note, and particularly those in which low morning temperatures alternate with correspondingly high evening temperatures; then also those in whom there is evidence of active involvement of the pleura; further, all cases of pregnancy, diabetes, and epilepsy, heart and kidney lesions, occurring in tubercular subjects, while a tendency to hemorrhage does not in itself constitute a contra-indication. If, moreover, every injection is followed by a marked reaction, and it is impossible to obviate this, either by a suitable diminution of the dose, or by using one that is larger, after giving the organism time to recover from the last reaction, it is evidently not advisable to continue the treatment. Generally speaking, Wright's method, or that of Wolff-Eisner, should be employed in those cases in which we are in doubt whether or not to use tuberculin at all. In fine, I would add that in surgical tuberculosis the physician should never withhold recognized surgical treatment, hoping that immunization treatment alone will suffice.

Reactions.—The reactions which follow the use of tuberculin for curative purposes are essentially the same as those which are noted when the material is injected for diagnostic reasons (which see). There are, however, certain points of difference. Generally speaking the reactions develop after a shorter time, which varies with the size of the dose. Following the injection of 3 to 20 milligrams there is frequently a response as early as eight hours, and after doses

of 50 milligrams this may even develop within four or five hours. The duration, moreover, is shorter, so that all the symptoms may have disappeared within eight hours, counting from the time of their development. The response, both local and systemic, moreover, is more intense, the former preceding the latter. As in connection with the diagnostic test, local redness develops at the point of injection after one or two hours; this is followed by pain and infiltration, reaching its maximum after about twelve hours and disappearing only after a number of days. The systemic response manifests itself in an initial chill or chilly sensations, headache, muscle pain, and fever. The reaction reaches its height after from four to twelve or fourteen hours (according to the size of the dose), and then subsides so that normal relations are restored within twenty-four or thirty-six hours, the patient merely experiencing a certain degree of lassitude and tendency to increased expectoration, which may continue for several days. The height of the temperature differs considerably, and while usually not exceeding 102° F., it may reach 103° and 104°.

In especially susceptible people, or when the higher doses are reached, the systemic symptoms may be much more severe and extend over many days, and during such a period it would, of course, be a mistake to repeat the injection. When these febrile periods are very lengthy and accompanied by lasting loss of weight and cardiac disturbance of notable degree, the treatment should be suspended or eliminated.

Results.—So far as the results of the tuberculin treatment go, so much depends upon the individual case, the duration of the disease, the character and seat of the lesion, the possibility of supplementing vaccination with adequate hygienic treatment, etc., that from a prognostic standpoint every case must be judged upon its own merits. Suffice it to say that the average case, *ceteris paribus*, does better under immunization treatment than without it, and that every physician should recognize the rationale and value of the method. But I feel very strongly that in order to obtain the best results the treatment should be carried out either in special institutions or by men who are thoroughly familiar with the intricacies of immunization methods. As a matter of fact the best results have been reported from just such sources.

Bandelier thus found that of 202 cases of pulmonary tuberculosis

which had been treated with tuberculin, 63 per cent. no longer had tubercle bacilli in their expectoration. The best results, as would be expected, were obtained during the first stage of the disease where 100 per cent. of the cases became bacilli-free; among those in the second stage this point was reached in 87 per cent., and among those in the third stage in 44.2 per cent. As Bandelier states, an equally favorable series has not been recorded in the literature. The patients in question had been treated in sanatoria belonging to the Landes-Invalidenversicherung, of Berlin. Koch's old tuberculin had been used in all cases where the physical examination suggested a tendency to fibrous changes, or in which, in spite of extensive infiltration, there was little secretion; also in bone and glandular tuberculosis and in tubercular fistulæ of the anus. Otherwise, *i. e.*, when there was extensive softening, or when febrile reactions would have been undesirable, new tuberculin was employed. The outlined treatment with old followed by new tuberculin was mostly used in advanced cases, and seems to have furnished the best results, as gauged by the disappearance of the bacilli in thirty-eight of sixty-nine cases, *i. e.*, in 55.07 per cent. Considering the advanced character of the lesion in these individuals, this is indeed quite remarkable.

If we contrast these findings with the results of a purely expectant (*sc.*, hygienic-dietetic) plan of treatment, where only 20 per cent. of the cases show loss of bacilli, no further argument in favor of the tuberculin treatment is required. It should be remembered, moreover, that the actual results were probably still better than is suggested by the above figures, if we consider that the improvement continues for three or four months after the treatment is suspended. They might have been still better, as Bandelier suggests, if the limit of immunization, *i. e.*, the maximal dose of tuberculin had been higher than 10 milligrams, which had been chosen as standard.

Of late, systematic efforts have been made to improve the hygienic condition of the tubercular poor, and to give these also the benefit of the tuberculin treatment when living in their own homes. As a consequence the outlook for these unfortunates has been materially improved. Friedrich thus records that of 700 cases of early tuberculosis which were treated in this manner the disease was arrested or the patients much improved in 51 per cent. of the cases. Similar results have been reported from other sources.

ESTIMATION OF THE OPSONIC CONTENT OF THE BLOOD (WRIGHT'S METHOD)

Before concluding this chapter it may not be out of place to give a brief account of the technique which Wright recommended for the purpose of estimating the opsonic index, but which at present has but little more than historical interest, insofar as its bearings on treatment or diagnosis are concerned. The necessary apparatus is pictured in the accompanying illustration (Plate III). It consists of a pipette (*a*) for collecting corpuscles; (*b*) a tube to receive the blood to be examined, in place in which the blood capsule (*c*) can also be used; and capillary pipettes (*d* and *e*) provided with rubber nipples. For purposes of incubation a special thermostat is recommended, but in its absence the usual laboratory incubator may be employed. The actual "reagents" are represented by the patient's serum, a normal control serum, washed leukocytes, and bacterial emulsions.

Preparation of the Patient's Serum.—A small amount of blood (about 6 or 8 drops) is collected in a little tube like the one pictured in Plate III at *b*, by puncturing the lobule of the ear at its free margin, in the usual manner, and dipping up the blood as it is milked out by moderate pressure. The little tubes measure about two inches in length and have a diameter of one-quarter of an inch; they may be closed with a little stopper or with adhesive plaster, and can then be readily transported. The blood is allowed to clot, the coagulum separated from the walls of the tube by means of a platinum wire, and the specimen centrifugalized until the corpuscles have been packed down and well separated from the serum.

In place of the tubes just described, which are really most convenient, Wright employs special capsules like the one pictured in Plate III at *c*, both ends of which are sealed. The blood is collected by puncturing the thumb near the root of the nail, after having previously allowed the arm to hang down and then applying some constriction behind the distal joint (tape, rubber tubing). The puncture is made with the sharp point (*s*) of the straight limb of the capsule. The sealed tip (*m*) of the bent limb is knicked off and the open end held to the exuding drop of blood which enters by capillary attraction until it reaches the mark *n*. After knicking off the sealed

tip at *s*, the capsule is inverted, when the blood will occupy the space above *s*. The aperture at *s* is again sealed, and the serum now separated from the corpuscles by centrifugation, to which end the capsule is suspended on the rim of the centrifugalizing tube by the bent limb. In the end the tube is cut with a file at *n*.

Preparation of the Normal Control Serum.—This is collected in the same manner as the patient's serum and separated from the corpuscles by centrifugation. It is best to pool three or four normal sera, viz., to mix equal quantities from three or four individuals. If, however, the serum of one single person (the experimenter, for example) has been thoroughly studied and always found normal, this single serum may suffice for ordinary purposes. Women during menstruation, hard workers, and individuals who are pale and below weight, even if otherwise healthy, should not be taken as controls, nor even included in a pool. Occasionally, apparently normal individuals are also encountered, who habitually have a higher opsonic content than normal, and such must, of course, also be excluded. The process of digestion further tends to increase the opsonic content of the blood, so that it is advisable to take the blood of the patient and the pool approximately at the same hour of the day. As with the patient's blood the control serum also should not be more than twenty-four hours old.

Preparation of Washed Corpuscles (Leukocytes).—The blood is most conveniently collected from the ear and received in a tube containing 1.5 per cent. sodium citrate in 0.9 per cent. salt solution. The amount will depend upon the number of specimens that are to be prepared; 1 c.c. is sufficient for at least a dozen mounts. Small test-tubes of 5 c.c. capacity are very convenient. Clots must be avoided and the specimen promptly discarded if the slightest coagulum forms. Wright lets the blood drop directly (from the finger) into the citrate solution, while I use the small tube *a* (Plate III) to make the transfer. To prevent clotting I use a little beaker with citrate saline, and between transfers always rinse the pipette in this and keep some of the solution in the end, so that the blood immediately comes in contact with this; a number of drops of blood are allowed to enter by capillary attraction and are then blown out into the little test-tube; after every addition the citrate tube is closed with the finger and inverted so as to secure uniform dilution. The corpuscles are then thrown down by centrifugation, the super-

natant fluid pipetted off and replaced with 0.9 per cent. saline, the corpuscles brought into suspension and again thrown down, when the saline is carefully withdrawn with a capillary pipette. Wright then uses the superficial layer of corpuscles only, as this is especially rich in leukocytes (the leukocytic cream).

If large quantities of leukocytes are required, rabbits are injected into the pleural cavity with 5 to 10 c.c. of an emulsion of aleuronat mush in bouillon, or 0.9 per cent. saline, the mixture being sterilized; killed cultures (at 120° C.) of staphylococci (albus or aureus) may be used for the same purpose. The needle for injection should be somewhat dull, so that bloodvessels are not injured and hemorrhage is prevented. After twenty hours the pleural cavity will contain an exudate rich in leukocytes, which is pipetted off, placed in citrate-saline, and washed as described above.

Ordinarily the leukocytes should not be kept longer than five or six hours.

Preparation of Bacterial Emulsion.—As the Wright technique necessitates working with uniform emulsions, *i. e.*, with emulsions in which the bacteria are evenly distributed, this step is really the *cruce* of the whole process. With certain organisms, such as the staphylococci, the difficulty is not so great, but with others, notably the tubercle bacillus, it is almost impossible to obtain uniform results.

Staphylococci and streptococci may be grown on plain agar, while gonococci, pneumococci, and meningococci are cultivated on blood agar or hydrocele agar. Small tubes, like the one pictured at *b* (Plate III) are charged with a little saline (0.85 to 1.2 per cent.). A bit of the culture is removed with a platinum loop and gently rubbed against the wall of the tube, at the surface of the liquid, until a uniform turbidity results throughout the specimen. This is then centrifugalized for a minute or two, so as to remove clumps as far as possible, and to obtain the desired degree of density of the bacterial emulsion. This point can only be learned by experience. For convenience' sake, small glass capsules may be prepared containing emulsions of barium sulphate of varying degrees of turbidity, and corresponding to bacterial emulsions of standard strength. With these the centrifugalized specimen may be compared before use in the actual experiment. Wright advocates an emulsion of cocci of such strength that with normal serum the average number of organisms per leukocyte (see below) is about four or five.

It has been recommended that the cultures should not be more than twenty-four hours old. This, however, is not necessary for all organisms. Knorr has shown in my laboratory that the same degree of phagocytosis is obtained with cultures of the staphylococcus more than a month old, as with young cultures. In the case of the typhoid and the colon bacillus, Wright recommends the use of cultures only four hours old, as with older cultures the resultant spherulation of the organisms is such that approximative results only can be obtained.

In the case of the tubercle bacillus, Cole obtained the best results by starting with living cultures on glycerin agar, which had been killed by exposure to sunlight for twenty-four hours. Some of the material is then scraped off, ground up in an agar mortar with 1.5 per cent. saline and centrifugalized to remove clumps. Cole states that if contamination is guarded against the supernatant fluid may be used for at least a month. I have not had occasion to use emulsions prepared in this manner, and am familiar only with emulsions made from dead and ground-up bacilli. A small quantity of this material is placed in an agate mortar and thoroughly triturated with 1.5 per cent. saline, which is *slowly* added drop by drop. The resultant emulsion may be freed from coarser clumps by centrifugation, but the smaller ones are practically impossible to remove. I have worked with heated and unheated, with extracted and non-extracted bacilli, with 0.1 and 1.5 per cent. saline, but I have not yet seen an emulsion of tubercle bacilli that was uniform.

In the case of the tubercle bacillus, Wright recommends that the emulsion should be of such strength that in the actual experiment one or two bacilli only are found on an average in each cell.

The Experiment Proper.—Having prepared the patient's serum, normal control serum, washed corpuscles, and the bacterial emulsion, these "reagents" are placed in a small rack, or in a dishful of sand covered with a piece of white filter paper, perforated to receive the tubes, and marked accordingly.

Mixing pipettes (Fig. *d* or *e*, Plate III) are prepared from glass tubing having an outside diameter of approximately 6 mm. To this end pieces of tubing are cut, measuring about 15 cm. in length, heated in the middle in the flame of a Bunsen burner until soft, and then drawn out after removal from the flame, so that capillary stems are obtained about 10 to 15 cm. long, with a diameter of from

0.5 to 1.0 mm. The ends are cut off square with a fine file. The tubes are marked about 1 to 2 cm. from the ends with a glass pencil and before use provided with medicine-dropper rubber nipples. One volume of the leukocytic "cream" (see Preparation of Leukocytes, above) is then drawn up to the mark, followed by one volume of serum and one of the bacterial emulsion, the three portions being separated from one another by little bubbles of air (see Fig. *d*, Plate III). The contents of the tube are next blown out upon a slide by gentle pressure upon the rubber nipple, well mixed by drawing them up and down in the capillary tube, then taken up in solid column, and the end sealed in the burner. The tubes are finally incubated for fifteen minutes at body temperature, which may either be done in an ordinary incubator or in a special "opsonifier."

After incubation the ends of the tubes are pinched off, drops are mounted upon *clean* slides, and after having been well mixed by passage up and down in the capillary pipette, exactly in the manner in which the mixture was originally made, spreads on slides are prepared by the aid of the narrow edge of a second slide, as in the preparation of ordinary blood smears. After drying in the air the specimens may be stained with aqueous methylene blue, with some polychrome dye, such as Jenner's, Hastings', Wilson's, or Giemsa's stain, or with Borrell's carbol-thionin,¹ the specimens being fixed with absolute methyl alcohol, if aqueous stains are to be employed, while this is, of course, unnecessary in the case of alcoholic mixtures. Tubercle specimens are fixed by immersion for one minute in a saturated aqueous solution of mercuric chloride. They are then washed off in water, stained with steaming carbol fuchsin, washed with water, decolorized in 2.5 per cent. sulphuric acid, treated with 4 per cent. acetic acid solution to destroy the red cells, again washed in water, counterstained with 1 per cent. aqueous methylene blue, washed once more, and then allowed to dry.

The average number of bacteria per leukocyte (*phagocytic index*) is finally ascertained by going over at least a hundred cells, and the *opsonic index* then calculated by dividing the patient's phagocytic index by the normal, which is taken as unity. *Example*: Supposing

¹ A saturated solution of thionin in distilled water is precipitated with a 10 per cent. soda solution; the precipitate is collected on a small filter, washed twice with distilled water, and then dissolved in 5 per cent. carbolic acid solution (1 gram : 100 c.c.). The solution must always be filtered before use.

that with the patient's serum the average number of organisms per cell was 5 and with the normal serum 10; then from the equation $10 : 1 :: 5 : x$, it would follow that the opsonic index is 0.5.

When Wright's studies on the opsonins first appeared they attracted a great amount of attention. This was largely owing to the fact that the author attached a significance to his observations which, if justified, would have meant an enormous advance not only in the diagnosis of certain bacterial infections, but also in their treatment. I cite some of his more important diagnostic deductions:

1. Conclusions which can be arrived at when we have at disposal the results of a series of measurements (opsonic determinations):

(a) When a series of measurements of the opsonic power of the blood reveals a persistingly low opsonic power with respect to the *tubercle bacillus*, it may be inferred, in the cases in which there is evidence of a localized bacterial infection which suggests tuberculosis, that the infection in question is tuberculous in character.

(b) When repeated examination reveals a persistently normal opsonic power with respect to the *tubercle bacillus*, the diagnosis of tubercles may with probability be excluded.

(c) When there is revealed by a series of blood examinations a constantly fluctuating opsonic index the presence of active tuberculosis may be inferred.

2. Conclusions which may be derived at where we have at disposal the result of an isolated blood examination:

(a) When an isolated blood examination reveals that the tuberculo-opsonic power of the blood is low, we may—according as we have evidence of a localized bacterial infection or of constitutional disturbance—infer with probability that we are dealing with tuberculosis—in the former case with a localized tuberculous infection, and in the latter with an active systemic infection.

(b) When an isolated blood examination reveals that the tuberculo-opsonic power of the blood is high, we may infer that we have to deal with a systemic tuberculous infection which is active, or has recently been active.

(c) When the tuberculo-opsonic power is found normal or nearly normal, while there are symptoms which suggest tuberculosis, we are not warranted, apart from the further test described below, in arriving at a positive or a negative diagnosis.

The further criterion to which reference has been made in the preceding paragraph is the following:

When a serum is found to retain in any considerable measure, after it has been heated to 60° C. for ten minutes, its power of inciting phagocytosis, we may conclude that "incitor elements" (immune opsonins) have been elaborated in the organism either in response to auto-inoculations, occurring spontaneously in the course of tuberculous infection, or, as the case may be, under the artificial stimulus supplied by the inoculation of tubercle vaccine.

The above considerations apply also in the case of other bacterial infections, and in the examination of exudates as well.

As Wright regarded the opsonic index as an indicator of the degree of immunity which develops as the result of bacterial vaccination (which see), he advocated that the dosage and frequency of injection should be controlled by opsonic determinations. According to his teachings the injection of a dose of vaccine is followed by a decrease of the opsonins (*negative phase*), which is of variable degree and duration, according to the amount injected. This is followed by an increase (*positive phase*) coincidently with which there is a corresponding improvement in the patient's condition. The idea of proper vaccination, then, is to so gauge and interspace the different doses that a negative phase is obviated as far as possible and a "high tide" of increased opsonic content secured.

It would lead too far to discuss the teachings of Wright in any detail at this place; suffice it to say that nearly all investigators who have busied themselves with his technique have come to the conclusion that the unavoidable sources of error are such that accurate results cannot be obtained. As a consequence its application loses much of its *raison d'être*, and at the present time there are few outside of Wright's own circle who are influenced in either diagnosis or treatment by the opsonic index. But this failure does not in the least diminish the importance of the principle of bacterial vaccination, a principle which had, however, been firmly established long before the opsonins were discovered.

It would, of course, be most desirable to possess an index to dosage and frequency of injection in vaccination, but a consideration of what has already been said regarding the aggressive forces of the bacteria will at once suggest that even if it could be possible to estimate the "opsonic index" with accuracy, this alone would

scarcely be of much value in the treatment of infections. For unless we can influence the aggressive forces of the invading organisms and notably their capsule-forming power, the production of a high content of opsonins in itself would lead to nothing.

In conclusion, I would briefly call attention to the fact that in the early days of the opsonic "high tide" I advocated a different method of estimating the opsonins, which was based upon the principle of dilution, and I note with satisfaction that this principle is now utilized in practically all laboratories (outside of Wright's) in which opsonic studies are being carried on.

CHAPTER XIII

PASSIVE IMMUNIZATION

WHILE active immunization is the procedure *par excellence* to be employed for prophylactic purposes, or in the treatment of those infections which are characterized by a chronic course, the indications for passive immunizations are essentially afforded by the acute infections, the idea being that in these the necessary time may not be available for the formation of protective antibodies, or that these are not furnished in sufficient quantity by the infected organism itself. The plan, then, is to introduce these principles from without, either in the form of antitoxic sera, or of bacteriolytic-bacteriotropic sera, as the case may be. That such sera may at times be serviceable also for prophylactic purposes, goes without saying, but it is natural that their value from this standpoint should be limited. For whereas in the actively immunized organism the entire defensive mechanism is thrown into action, and *remains* in action, often for a considerable length of time, the protective principles which we introduce from without are after all limited in quantity, and are, no doubt, eliminated or destroyed after a relatively short time. If such sera are administered at a time, however, when the organism has just become infected, or is immediately threatened with infection, their use is unquestionably rational, and frequently of great value.

As regards the mode of action of the two types of sera, which are available for passive immunization, I would merely recall that those organisms which are strong toxin producers are also of a low grade of infectiousness, and that the macroörganism can usually overcome the infection as such without much difficulty, if it is protected against the harmful effect of the toxins. In tetanus the infection is indeed usually already under control before the toxins, which have been liberated, can exercise their fatal action. With the true parasites and semiparasites, on the other hand, where toxins either play no role or only a limited role, the bacteriolytic sera would, *a priori*, be expected to be of service, but, unfortunately, their actual

therapeutic value is very small. In our discussion of the different sera, we shall accordingly only afford a limited space to the latter, and largely confine our attention to those possessing marked antitoxic properties, the discovery of which ranks as one of the most important in the science of medicine. The sera which here enter into consideration will be discussed under the heading of the diseases against which they are directed.

ANTITOXIC IMMUNIZATION

DIPHTHERIA

After Roux and Yersin had shown that the clinical picture of diphtheria is due to the action of a soluble toxin which is secreted by the corresponding organisms (1888), v. Behring found that animals which have been immunized against diphtheria are thus rendered resistant to the toxin in question, and that the blood of such animals contains a principle which can be transferred to other animals and can protect these against subsequent infection, or cure this, as the case may be (1890). This principle he termed *antitoxin*.

The first attempt to apply this important discovery to the cure of diphtheria in the human being was made in Berlin in v. Bergmann's clinic (1891). The results, while suggestive, were not altogether satisfactory, however, as the serum which was then available was too weak and the dosage too small. But subsequent investigations by a number of different observers, notably Roux, Ehrlich, Kossel and Wassermann, Aronson and Baginsky, etc., supported v. Behring's claims in their entirety and demonstrated conclusively that one of the most fearful and most intractable diseases to which the human being is subject had indeed been conquered. Since then diphtheria antitoxin has been the means of saving untold thousands of lives which otherwise would have been doomed, and has thus proved one of the greatest blessings to the entire civilized world.

While people still die of diphtheria at the present day, this is largely owing to ignorance or indifference on the part of those to whom the medical profession must after all look for the earliest diagnosis of the disease, or at least for the recognition of those symptoms which should serve as danger signals, *i. e.*, the parents

and guardians of young children, and of those who are so situated that they cannot look after themselves. Of physicians, we may hope, there are none who at the present day would withhold from their patients what the scientific world has come to recognize as the most potent and important curative agent in the management of the disease in question. If, by any chance, however, there should be such a person, then the laity should realize that the non-use of diphtheria antitoxin, in the absence of special indications to the contrary, constitutes sufficient evidence of inefficiency on the part of the practitioner to warrant his prosecution in the courts.

Preparation of Diphtheria Antitoxin.—While the earliest attempts at immunization were made with the serum of some of the smaller laboratory animals (sheep and goats), it soon became apparent that from such sources a sufficient supply could not conveniently be secured, and at the present time the horse is universally employed as antitoxin producer.

The animals which are chosen for this purpose are first tested with tuberculin and mallein for freedom from tuberculosis and glanders, and, further, receive an injection of tetanus antitoxin to counteract any accidental infection of this kind which might accidentally occur during the period of time that the animals are furnishing antitoxin. They are well fed and groomed, and every effort in short made to maintain them in the best condition possible.

For purposes of immunization the toxin furnished by a special strain of the diphtheria bacillus is now used the world over. This strain has been studied with special care by Park and Williams (New York), whose names it bears, and is grown in 2 per cent. peptone nutrient bouillon of an alkalinity corresponding to 8 c.c. of normal soda solution per liter (above the neutral point to litmus), the broth being beef-broth, and the peptone the usual preparation of Witte. This medium is placed in comparatively thin layers in wide-mouthed Erlenmeyer flasks, and kept at a temperature of from 35° to 36° C. At the end of a week the toxin production has reached its maximal point, when the cultures are tested in reference to their purity, and are killed off by the addition of 10 per cent. of a 5 per cent. solution of carbolic acid. After standing for forty-eight hours, most of the bacilli have settled to the bottom, the clear supernatant fluid is filtered through sterile filter paper and is stored in full bottles in the refrigerator. Before use it is tested on guinea-

pigs. If the material contains an adequate amount of toxin, less than 0.01 c.c. should kill an animal, weighing about 250 grams.

Since the injection of the crude toxin often gives rise to quite severe local as well as systemic reactions, various attempts have been made to so modify the material as to diminish this feature as far as possible without interfering with its antigenic value. This has been accomplished, in a measure, by giving the horse a large dose of antitoxin mixed with the first three doses of toxin. In the laboratories of the Health Department of New York City the animals thus receive as initial dose an amount of toxin sufficient to kill 5000 guinea-pigs (average weight 250 grams), *i. e.*, about 20 c.c., and mixed with this 10,000 units of antitoxin. This injection (given subcutaneously) is followed by a febrile reaction which lasts for three to five days, when a second injection of a slightly larger dose is given, and after a similar period of time a third one, both being accompanied by a dose of 10,000 antitoxin units, as in the first instance. After that the immunization is continued with increasing doses of toxin, given by itself, and at intervals of five to eight days, until at the end of two months from ten to twenty times the original amount is given (Park). If during this period the animal should at any time react unduly by fever, or if any infiltration should occur, it is recommended to resume the combined administration of toxin with antitoxin, as in the beginning. At the expiration of six weeks or two months the animal's blood is tested for its content in antitoxin. If by that time this has reached a titer of 100 to 150 units the animal may be expected to ultimately furnish a serum of moderate strength. If high-grade sera only are desired, it is needless to continue with any animal that at this period does not give a titer which is higher than 150.

After this test-bleeding, immunization is further continued with increasing doses, at intervals of three days to a week, until the animal furnishes a serum with the titer that is desired, or until this can no longer be increased. At the end of three months two or three animals out of fifteen or twenty will give a titer of about 500 units, and half of the total number one of 180 to 200. Further injections may increase the production still further, but it is noteworthy that values of 500 to 600 units are rare. Higher values than 1000 are very uncommon, and Park states that of his horses not a single one ever yielded 2000 units. In those animals, moreover,

which do yield exceptionally high values, the high tide of antitoxin production is only of brief duration. As the maximum production of antitoxin even under the most favorable conditions does not continue beyond a few months, and is then followed by a decline, in spite of further immunization, it is advisable to give the animals a period of three months' rest in every twelve that they are in service. If this is done, the best horses furnish high-grade serum during their periods of treatment for from two to four years (Park). As 6000 c.c. of blood may be taken from an animal at intervals of one month, it will be seen that the yield per year amounts to from 36 to 54 liters, allowing for the three months' trial immunization during the first year and three months of rest.

When it is desired to draw off some of the blood a superficial vein of the neck is punctured with a fair-sized, sharp-pointed cannula and the blood allowed to flow through an attached tube into large Erlenmeyer flasks, special pains being taken to work aseptically throughout the whole procedure. The flasks are placed in a slanting position before the blood-clots, and kept in a cool room for three or four days, when the serum which has separated out is pipetted off and stored, preliminary to a bacteriological examination and the determination of its titer, after which it is filled into little ampoules, or into individual syringes, as the case may be, and is then ready for use. In Germany, carbolic acid is used as a preservative, to the extent of 0.5 per cent., while in the United States, 0.4 per cent. tricresol is preferred, unless indeed the serum is used as such, which is now frequently the case. The individual package is appropriately labelled, and the date indicated, after which it should no longer be used. This is necessary, as the antitoxic titer diminishes in the course of time. Park states that the serum which is prepared by the New York Board of Health remains within 10 per cent. of its original strength for at least two months, when kept from the access of air and light in a cool place, but that within a year the loss in strength may amount to 40 per cent.

Determination of the Titer.—In the study of the titer of diphtheria antitoxin the following *standards* are employed: *As unit of diphtheria toxin* we designate that quantity expressed in fractions of a c.c., which is just sufficient to kill a guinea-pig weighing 250 grams in the course of four or five days. In other words, the single lethal dose constitutes the unit.

A toxin broth which contains 100 units per c.c., v. Behring has termed a *normal toxin solution*, and he designates this by the formula DTN, M_{250} , which signifies: diphtheria toxin, single normal, in reference to a guinea-pig weighing 250 grams. Of this 1 c.c. would suffice to kill one hundred guinea-pigs and 0.01 c.c. a single animal. A double normal toxin solution, DTN_2M_{250} , would accordingly be one of which one-half that dose, *i. e.*, 0.005 c.c., would suffice to kill a guinea-pig of standard weight. A *unit of antitoxin*, on the other hand, is that quantity which is capable of neutralizing 100 units of toxin, and we designate as *normal serum* one of which 1 c.c. will neutralize 1 c.c. of normal toxin solution, *i. e.*, 100 units of toxin.

1 c.c. normal antitoxin serum = 1 c.c. normal toxin solution is sufficient to protect 100 guinea-pigs, each against a single lethal dose—0.01 c.c.—of normal toxin).

Ehrlich designates as L_{\dagger} (limes = limit) that quantity of toxin which when mixed with one unit of antitoxin and injected subcutaneously into a guinea-pig weighing 250 grams will kill the animal in four or five days, while he denotes the quantity which is just neutralized by 1 unit of antitoxin, and which will hence not kill the animal when injected together with the toxin as L_0 .

To determine the strength of a given serum it is for practical purposes only necessary to inject a series of guinea-pigs subcutaneously, each with a mixture containing say 100 units of toxin and varying quantities of the serum under consideration. If the animal dies within the first days the amount of antitoxin was evidently not sufficient to neutralize all the toxin, and the serum hence had a titer lower than 1 unit to the c.c. If death takes place on the fifth or sixth day the antitoxin content is just a unit, and if the animal does not die at all, it must have been stronger than this. Supposing that 1 c.c. of the serum in a dilution of 1:1000 had been sufficient to delay death until the fifth or sixth day, then 0.001 c.c. of the concentrated serum would represent one antitoxin unit, and its actual titer would hence be 1000 units per c.c.

In Germany the production of antitoxin is carefully supervised by the government and every preparation tested in the Institute for Experimental Therapy, of which Ehrlich is the head. In the United States there are now also stringent laws regulating its preparation, and specimens are purchased from time to time in the open

market for examination at the hygienic laboratories of the Public Health and Marine Hospital Service.

In the United States diphtheria antitoxin is now marketed in 500, 1000, 2000, 3000, 4000, 5000, and 10,000 unit doses.

The Injection.—The injections are usually given into the loose subcutaneous tissue between the shoulder-blades, into the abdominal walls, or into the district overlying the triceps. The skin should be scrubbed with soap and water and then with alcohol, or as is now also advised, merely painted with tincture of iodine about the point of injection. If a separate syringe be used this should, of course, be sterilized by boiling, but in the United States the manufacturers now send the antitoxin out in separate syringes which are already sterile and ready for immediate use.

Of late it has been suggested that a more powerful effect may be secured if the antitoxin is administered intramuscularly, or, still better, intravenously. To this there can be no objection if the amount of preservative that is thus injected at one time remains within the limits of the permissible dose. In Heubner's clinic 18 c.c. of serum containing 0.5 per cent. carbolic acid have thus been injected at one time and the dose repeated within twenty-four hours. With us, in the United States, where no preservative is frequently used, even this objection does not exist. The advantage of the intravenous over the subcutaneous method of administration has been clearly shown by Berghans, who found in the animal experiment that whereas 40 units of antitoxin were necessary to prevent the death of a guinea-pig when given subcutaneously, 0.08 was sufficient when injected directly into the circulation, the amount of toxin having been the same in both instances. The importance of resorting to this method of administration is further emphasized by the observation made in the Danish Serological Institute that following the subcutaneous use of the antitoxin this does not reach its maximum in the circulation until the second or third day. Eckert thus very properly insists that the intravenous method is the method *par excellence* to be employed, and that with its general adoption the death-rate from diphtheria will be lowered still farther (see below).

Dosage and Uses.—In the treatment of diphtheria by antitoxin it is important to bear in mind that the quantity of toxin that is produced and likely to be absorbed is, *ceteris paribus*, the greater the longer the duration of the disease, and that the union of the

toxin with the receptors of sensitive cells will be the firmer the longer this has lasted. It follows that large doses will be required, if the patient first comes under observation after the disease has already existed for a number of days, and that in the presence of toxic symptoms, indicating that toxin has already been anchored by sensitive cells, very large doses only can be expected to be helpful. It is accordingly recommended that the physician should not delay the use of antitoxin until a bacteriological examination has been made, but to resort to it whenever diphtheria is suspected. This rule is indeed the only natural one to follow.

As to the size of the initial dose, the last word has probably not yet been spoken. In the earlier days of antitoxin treatment 100 to 200 units were recommended, but since then there has been a tendency to ever-increasing amounts, and in the United States 3000 units may now be regarded as an average dose in cases of moderate severity. If a longer interval than twenty-four hours has elapsed before the patient is first seen, the dose should be still larger, and if threatening symptoms of any kind exist the physician should not hesitate to inject 10,000 units or more at the time of his first visit. Some writers, indeed, have used much larger amounts (up to 50,000 to 100,000 units) and have reported favorable results in the most desperate cases.

Following the first injection the antitoxin is continued at intervals of twelve to twenty-four hours, until the disease is evidently under control, and I would emphasize once more that much time may be saved if the injections are given intravenously, or even intramuscularly. In severe cases the subcutaneous administration should unquestionably be abandoned, since the absorption owing to the lowered blood pressure must then be still slower than in a healthy individual, where the maximal blood content in antitoxin is scarcely reached before the third day.

Total Quantity that may be Administered.—As to the quantity of antitoxin which *may* be administered in the course of the malady there is apparently no limit. Bankier thus reports the case of a child in which 72,000 units were given, and in which recovery occurred in spite of the most ominous symptoms (profuse nose-bleed, extensive hemorrhagic ecchymoses of the skin, paresis of the pharyngeal muscles, of the palate, of the larynx and some of the skeletal muscles, nephritis with edema, etc.). Gabriel, at Neisser's clinic,

gave 4000 to 5000 units every five days for four weeks, in severe cases. At the Berlin Charité four-fifths of the cases require from 1500 to 4500 units; in the remainder 9000 to 18,000 are common amounts, and in the severest cases 30,000 to 65,000 have been used. Above all, then, the physician should not despair in the face of a grave case, but use the antitoxin systematically until the child is either dead or out of danger. The same rule applies in the management of those cases in which post-diphtheritic paralyses have occurred. In the past it was thought that antitoxin would be of no avail in such cases, but in the light of more recent experience it would seem that here also much good may come from the systematic use of the serum.

The question, of course, suggests itself, why antitoxin should still be of service when once the toxin has been anchored to sensitive receptors; but I would recall that as a consequence of immunization, the specificity of the receptors for the corresponding antigen may be very materially increased and that the toxin in question will hence have a greater affinity for the antitoxin furnished by the horse than for the sessile receptors of the patient. Hence, the possibility exists, theoretically at least, that an active antitoxin may be able to break the combination between the toxin and the patient's receptors, and our clinical experience suggests that this actually occurs. To effect this end, however, large doses are evidently necessary.

Prophylactic Dose.—For prophylactic purposes a dose of from 500 to 1000 units has been found sufficient to afford protection for approximately three weeks (see curve above). Whether or not this period could be lengthened by the administration of larger amounts seems doubtful, in view of the fact that the drop in the blood content of antitoxin which takes place within the first few days of its injection is the more abrupt the larger the dose. This will be understood if we bear in mind that the antitoxic properties of horse serum are intimately connected with its globulins, and that these are alien albumins which the body cannot utilize as such and which it accordingly tries to destroy as soon as possible.

Contra-indications to the Use of Antitoxin.—In view of the fact that a small number of people are hypersensitive to the use of horse serum to such a degree that a *first* injection even may be followed by most alarming symptoms, and in rare instances by death, some physicians have of late hesitated to use antitoxin as promptly as has generally

been urged. Should such symptoms develop, it is recommended to administer atropin and adrenalin hypodermically and to resort to artificial respiration. It should be borne in mind, however, that actual disaster is an extreme rarity when compared with the innumerable instances in which antitoxin is used without any untoward results, and that the danger which the unprotected patient incurs from the diphtheria is infinitely larger than that which would likely follow the use of the serum. Unless, therefore, it is known beforehand that the patient is hypersensitive to such an extreme degree, there should be no hesitancy on the part of the physician to use the serum.

It would, of course, be ideal if some method could be worked out which would enable us to definitely establish the existence of abnormal hypersensitiveness before the injection, but as yet no such method exists. In some instances in which alarming symptoms followed the injection of the horse serum a history was obtained that the patients had been subject to asthmatic attacks, and in some of these such attacks were brought on when the individual came into close contact with horses. It would accordingly be well to inquire into this point before the injection is given, and possibly to rule out from the treatment all those in whom a distinct history of asthma is obtained. In such cases antitoxin derived from some other animal than the horse could probably be used with impunity, and it is urgently to be hoped that ere long the manufacturers will place such material upon the market.

This could then also be employed in those cases in which horse serum has been used not long before, and in which we would hence have reason to expect the development of a sharp attack of serum sickness. The nature of the latter we have already discussed before (Chapter XI), suffice it to say at this place that its development cannot be regarded as a contra-indication to the use of the serum, and that not a single case has been reported in which the serum sickness in itself has endangered the life of the patient or caused any permanent damage to the individual. That it is undesirable, of course, stands to reason, and as the liability to the disease increases to a certain extent with the amount of the serum employed, it follows that sera of high potency in small bulk are generally to be preferred to larger quantities of serum of low antitoxic content. As the blood of adults, moreover, has been found to contain not inconsiderable amounts of natural diphtheria antitoxin, the use of

horse antitoxin is less urgent in these for prophylactic purposes than in children and can indeed often be neglected.

The Avoidance of Anaphylaxis by the Production of Antianaphylaxis.—Of late the suggestion has been offered that it may be possible to produce a state of antianaphylaxis (which see) by injecting the patient with a small quantity (0.5 c.c.) of antitoxin (sc., horse serum) a few hours before the principal injection is made, and that any dangers arising from anaphylaxis may thus be minimized or altogether eliminated. This should be borne in mind if serum treatment is necessitated in a patient who has been previously injected with horse serum. That antianaphylaxis may indeed develop in a very short time following the introduction of serum is undoubted, and Friedberger has lately devised an apparatus by means of which an intravenous injection of serum may be given so slowly that antianaphylaxis has an opportunity to develop during the administration.

Results.—If now we come to study the effect which the treatment of diphtheria with antitoxin has had upon the mortality of the disease, it is apparent from a survey of the accompanying table that the lowest death-rate will be obtained, if the injections can be given on the first day, and that the mortality percentage increases for every day that the treatment is delayed. Taking the results corresponding to the first day we have an average of 4.8 per cent. Further argument than this should be unnecessary to convince anyone that in the use of antitoxin we now have a weapon in the face of which diphtheria has indeed lost its terrors, and that a *physician who refuses to avail himself of its use is indeed unfitted to practise his profession.*

Author.	No. of cases.	Mortality per cent.	First day.	Second day.	Third day.	Fourth day.	Fifth day.	Sixth day.	Later than sixth.
Welch	1489	14.2	2.3	8.1	13.5	19.0	29.3	34.1	33.7
Hilbert	2428	18.3	2.2	7.6	17.1	23.8	33.9	34.1	38.2
Collective report of American Pediatric Society	5794	12.3	4.9	7.4	8.8	20.7	35.3		
Austrian collective report	1103	12.6	8.0	6.6	9.8	25.5	28.8	30.7	21.0
German collective report	9581	15.5	6.6	8.3	12.9	17.0	23.2	...	26.9

In the earlier days of the use of antitoxin the question was asked whether the lower mortality could not be explained on the assump-

tion that the diphtheria epidemic which was then prevailing was of an unusually mild type. We know as a matter of fact that the "virulence" of a disease undergoes periodic fluctuations, so that there is some reason in such a suggestion. But even so, the low mortality, when treatment was instituted on the first day, which was early noted, should have been sufficient to dispose of this possibility, for up to that time no treatment that had been previously in use could boast of such a result. But aside from this there are many other observations which prove beyond a shadow of a doubt that the low general mortality from diphtheria is really due to the use of antitoxin and not to accidental factors. At the Blegdam Hospital of Copenhagen during an entire year all diphtheria patients admitted on alternate days were thus treated with antitoxin, while those entering on the intervening days were given no serum. The result was the following:

Of 204 cases without croup treated with serum 5 died, giving a mortality of 2 per cent.

Of 210 cases without croup treated without serum 14 died, giving a mortality of 7 per cent.

Of 35 cases with croup treated with serum 3 died, giving a mortality of 8 per cent.

Of 43 cases with croup treated without serum 15 died, giving a mortality of 35 per cent.

Evidence of the same kind is afforded by the observation that during the year 1894 in Heubner's clinic the mortality had been lowered to 23.08 through the use of antitoxin, while in another hospital in the same city where no antitoxin was as yet available, the death-rate was 43.36 per cent. Körte further reports that in the days preceding the introduction of the serum the death-rate among the tracheotomized children in his clinic was 77.5 and subsequently 52.4. Similar figures were obtained by Siegert in his collective report based upon an analysis of 30,369 operated cases of diphtheritic larynx stenosis; of these 17,499 belonged to the pre-serum time and furnished a death-rate of 60.38 per cent., as contrasted with a mortality of 36.32 among 12,870 cases that had been treated with antitoxin. These figures speak for themselves.

The question, of course, suggests itself, whether it should not be possible to abolish the death-rate from diphtheria altogether, if once all cases could be treated with antitoxin on the first day of the

disease. As a matter of fact there are physicians who have not a single death to record among just such cases, even though their experience is based upon a fairly large number of observations. Nevertheless, there are instances where the injections have been started in time and in which death nevertheless occurred (see table above). Whether any of these could have been saved by injecting the antitoxin intravenously or by using larger doses is now, of course, impossible to say, but the possibility unquestionably exists. But even so we should remember that our serum is after all purely antitoxic in character, and that unless the body can successfully destroy the infecting organisms the battle may yet be lost, and it is this factor which may be responsible for the number of deaths that yet occur, even though the antitoxin be used at the very start. To overcome this possible obstacle to a zero mortality it would be tempting to use a corresponding vaccine simultaneously with the antitoxin. This has indeed been advocated by several investigators and deserves serious consideration. Petruschky records that he has succeeded in freeing bacillus carriers in this way of their dangerous guests.

TETANUS

The preparation and titration of tetanus antitoxin is based upon practically the same principles as that of diphtheria antitoxin, which we have considered in some detail in the foregoing section. The standards employed in Germany are the following:

One *unit of toxin* is that quantity which is capable of killing 4,000,000 white mice (of an average weight of 10 grams each) within four or five days with the characteristic symptoms of tetanus.

A toxin solution of such strength that 1 c.c. contains one unit of toxin is designated as *normal toxin*.

One *unit of antitoxin* is that quantity which will protect a mouse weighing 10 grams against 4,000,000 fatal doses of toxin, when injected subcutaneously.

A *normal antitoxic serum* is one of which 1 c.c. contains one unit of antitoxin.

In the United States an official standard unfortunately does not yet exist, and as the standards of the different manufacturers are not alike, physicians are practically obliged to express their dosage in terms of cubic centimeters rather than in antitoxin units.

Von Behring's fluid antitoxin is marketed in 100 unit (10 c.c.) and 20 unit (2 c.c.) doses; in addition to this a solid antitoxin, of which 20 units represent a dose, is also available.

Dosage and Uses.—For prophylactic purposes, 20 units (2 c.c.) should be injected about the site of injury, and if large nerve trunks have been exposed, in part into their substance: the idea being to bind the toxin which is formed about the point of infection, before it leaves this district, which takes place along the lymphatics and the nerve fibers. At the same time, it is recommended to give a subcutaneous injection of 100 additional units (10 c.c.) at an indifferent point, and to repeat the dose within six to eight weeks, as the immunity which is afforded only lasts for that length of time.

When symptoms of tetanus already exist, very little is to be expected from the use of the antitoxin for the reason that these symptoms indicate that a union with sensitive receptors (in the central nervous system) has already occurred, and that the antitoxin cannot penetrate to those points from intact bloodvessels. Neither the subcutaneous nor the intravenous route hence offers much hope of a satisfactory result. The attempt has accordingly been made to bring the material into immediate contact with the central nervous structures, by intraneural injections, through intracerebral injections and by its introduction into the subarachnoid space. The intracerebral method is to be deprecated altogether, as the death-rate following its use has been exceedingly high. More appropriate is the intraneural route, to which end the larger nerve trunks, along which absorption has likely taken place, must be exposed and injected at different points in their course. Unfortunately, not much serum can be introduced in this manner, and it is natural that the patient should subsequently suffer a good deal from the resulting neuritis. By the subdural route, on the other hand, it is easy to introduce large quantities of serum, and as Stintzing and Küster have already demonstrated that the cerebrospinal fluid usually contains a considerable amount of toxin in human tetanus, this method of treatment seems rational and likely to do good so long as recovery is at all possible, *i. e.*, so long as the union between toxin and the sensitive receptors is still capable of being broken. It is recommended to tap the subarachnoid space in the usual manner, to allow as much of the meningeal fluid to escape as possible, care being taken, however, not to let the pressure fall too low, and then to

slowly inject an equivalent volume of serum (10 to 20 c.c.) at a rate of about 2 c.c. per minute. According to the requirements of the case, this may be repeated several times within the same twenty-four hours, and continued on the following days.

As antitoxin treatment in tetanus can be expected to do good only so long as the toxin has not combined with the sensitive receptors of the central nervous system (barring those exceptional cases where this union can still be broken), and so long as it can be readily reached by the antitoxin, *i. e.*, before it has begun its travel along the axis-cylinders of the affected nerves, it follows that its use must be largely limited to prophylactic purposes. As the treatment, however, is of signal value, when employed to this end, the practitioner should resort to its use in *all* those injuries which are likely to favor infection with tetanus bacilli. It is hence recommended in connection with all wounds which have been contaminated with earth, manure, decomposing vegetable matter of any kind, particles of clothing, especially in puncture wounds, such as those produced by splinters of wood, rusty nails, and broken crockery; then in connection with all wounds caused by exploding fire-arms, cartridges, fire-crackers, rockets, in wounds caused by unclean instruments, as on battle fields, after division of the umbilical cord, removal of the placenta, etc. In all such cases the use of tetanus antitoxin is strongly to be advocated, and should become a uniform practice.

When once tetanus symptoms have developed, very little can be expected. If the attempt is to be made, however, it should not be delayed unnecessarily, and the subdural route chosen by preference. When large nerve trunks have been exposed, intraneural injections should be given in addition, besides which subcutaneous injections also may be employed. Intravenous injections, as I have already pointed out, can hardly do any good.

Results.—If now we turn to an analysis of the results which the introduction of the antitoxin treatment has produced, we may practically confine our attention to the prophylactic side of the question. The evidence here is quite conclusive that its timely use may be the means of saving many lives. In our own country, where the anniversary of the birth of the nation's independence has in the past been annually celebrated by a tetanus orgy, the death-rate in the absence of prophylactic treatment has been perfectly appalling. Liell, in an analysis of 350 cases, thus reports that of this number

only seven recovered (mortality 98 per cent.), of which five had received the prophylactic treatment. Scherk then mentions that of 591 cases of Fourth-of-July injuries which received prophylactic injections of antitoxin, not a single one developed the disease. Equally convincing are the reports from certain hospitals, in which antitetanus injections are given as a matter of routine in all cases where contamination of wounds with dirt from the street has occurred and where the disease is under these conditions hardly ever seen.

While tetanus is a fairly common malady in the province of Pommern it has thus been noted at the surgical clinic of Greifswald, where the prophylactic treatment of all primary injuries has been carried out for a number of years, that tetanus among the injected is practically unheard of, while it is common enough among patients that are sent in from the surrounding districts where this treatment is not in use. In Indo-China further, where formerly one-fifth of all newborn children succumbed to tetanus of umbilical origin, Calmette found that the administration of the dried preparation to the stump of the umbilicus was sufficient to prevent the outbreak of the malady. Quite suggestive also are the results which have been obtained in veterinary practice. Nocard thus reports that in a certain quarter of Paris where tetanus was exceedingly common among horses, not a single case developed among 2727 injected animals concerning which he received reports, and of which 2300 had been castrated; while during the same period of time there occurred 259 cases among those that had not been protected.

Evidence of this sort is now so abundant that the importance, nay the necessity, of prophylactic treatment in the injured, where there is the slightest reason for anticipating the possible development of tetanus, cannot be too strongly urged. When a physician nowadays quietly dresses an extensive scalp wound of the head, which has been freely contaminated with manure, and does not give his patient the benefit of a prophylactic injection of tetanus antitoxin his negligence is certainly but little short of criminal.

The question may, of course, be asked, whether tetanus never develops if an early injection of antitoxin be given. While we must admit that the protection is not absolute, the fact remains that if tetanus does occur under such conditions its course is very mild. Küster thus mentions a case where infection occurred accidentally in a laboratory with a highly virulent culture, and where, in spite

of prophylactic treatment, tetanus developed on the sixth day. In such a case ordinarily death would unquestionably have followed, but, as it was, the patient had an uncommonly mild attack which resulted in recovery.

Regarding the effect of the antitoxin treatment upon the malady when once this has developed, very little need be said. If we rule out from our consideration all those cases in which the first symptoms have developed after nine days or still later, we may say that death will result no matter whether the patient is injected or not. In the case of the remainder, we must remember that the patient's chances are the better the longer the period of incubation, so that the conclusion is not necessarily warrantable that recovery has taken place in such cases *because* of the injection. The best that we can say is that the treatment *may possibly* help, but that we cannot always logically attribute recovery to the treatment. It should be tried, but not too much should be expected.

DYSENTERY

While the attempts at prophylactic vaccination against infection with the Shiga-Kruse bacillus have not led to very satisfactory results (see p. 200), there is evidence to show that the use of the corresponding antiserum exerts a beneficial influence upon the course of the malady, when this has once developed. Regarding the mode of action of the antisera which were first prepared by Shiga and Kruse, there has been some controversy, it having been thought at first that their effect was essentially bacteriolytic in nature. Subsequently, however, when it was shown by Kraus and Doerr that the dysentery bacillus produces a true toxin, and that the same effect could be obtained with an antiserum, produced with this as antigen, the conclusion naturally suggested itself that the beneficial effects reached with the older preparations, where unfiltered cultures including the bodies of the bacilli represented the antigen, were probably also owing to contained antitoxins.

Preparation.—The preparation of antidysentery serum is conducted along similar lines as that of the other sera, which are used for passive immunization, horses being employed as the antibody producers. As in the immunization against diphtheria and tetanus

toxin a basic (Grund) immunity is first established by injecting a certain quantity of antiserum together with, or twenty-four hours preceding, the introduction of the toxin, or the toxin cultures, after which the process is continued with these alone.

Dosage and Uses.—The serum which is used for curative purposes in Vienna is of such strength that 0.1 c.c. at most will protect a rabbit weighing 1000 grams against a separate though simultaneous intravenous injection of a single lethal dose of the toxin. The curative dose of such a serum for the human being varies between 10 and 20 c.c., which may be repeated several times in severe cases. In extreme cases the French observers have used as much as 80 to 100 c.c. on the first day, and have repeated this on the following days. In three instances 240, 380, and 1080 c.c. were injected altogether, *i. e.*, doses which seem unwarrantably and unnecessarily large, if an active serum was really at hand. After the disease comes under control, as is evidenced by a diminution in the number of the stools, smaller doses may be given during the following days.

For prophylactic purposes the same dosage (10 to 20 c.c.) is recommended, and it is further advised to repeat the injections after two or three weeks, as the protection only lasts a short time. As the different manufacturers do not employ the same standards the practitioner must use the serum in accordance with the printed directions which accompany the individual package.

Injections.—The injections are given subcutaneously in the usual districts. As the Shiga-Kruse strains alone are toxin producers, while the Flexner type does not belong to this order, and as the serum corresponding to the former is markedly specific in its action, it is advisable to ascertain at the time of an epidemic whether the infection is actually of this type. Unless this is done it would not be warrantable to ascribe a lack of action to the serum when no effect is observed.

Results.—Regarding the results which have been obtained with the serum in question, it seems that the treatment is actually quite useful both for prophylactic and curative purposes, though adequate statistics are not yet available. More convincing than mere figures are the observations which have been made at the sickbed, by individual observers, all of whom speak quite enthusiastically of the marked effect of the injections upon the number of the stools, which frequently drops quite suddenly even within the first twenty-

four hours; then upon the pain and upon the general condition of the patient. Even in chronic cases much benefit may be expected. Veillard and Dopfer thus mention a case which had lasted five months, in spite of the most varied treatment, where recovery occurred after three injections of serum.

If we bear in mind that, next to typhoid fever, bacillary dysentery is probably the most formidable common disease with which military surgeons have to deal, it would suggest itself that in times of war, or when large bodies of men are concentrated within a narrow compass and are obliged to drink water of unknown quality, prophylactic treatment with antidysentery serum might prove of signal benefit.

CHOLERA

Although a number of different attempts have been made to produce an active antiserum for the treatment of Asiatic cholera, nothing of real value has as yet been accomplished. This is probably owing to the fact that while the symptom complex of cholera is evidently largely the result of an intoxication, the toxins in question are probably only in small part *true* toxins, but essentially endotoxins against which antitoxins are produced only to a slight extent, if at all.

The only preparation of this order which deserves any consideration, is the antiserum of Kraus, in the production of which the El Tor vibrio was used as antigen. This organism, it may be recalled, was obtained by Gottschlich in 1905 from the intestinal contents of pilgrims who had died at El Tor from dysentery, and is not identical with the true cholera vibrio, but evidently very closely related to it. But unlike the cholera vibrio, the El Tor furnishes a true toxin in fairly large amount, against which an active antitoxin can be obtained. This latter, according to Kraus, neutralizes the toxin of true cholera as well, and more efficiently than the antitoxin resulting from immunization with the latter. He has therefore recommended it for the treatment of Asiatic cholera. From the reports which have thus far been obtained it is, however, scarcely possible to reach a definite conclusion regarding its value. Ketscher and Kernig used the serum in 119 severe and moderately severe cases, with a death-rate of 58 per cent. in those who had received subcu-

taneous injections, and one of 50 per cent. when used intravenously; while the general death-rate among the non-injected cases was 63.4 per cent. Other observers contrast a mortality of 57.5 per cent. among the treated with one of 84.3 per cent. among the untreated cases. The verdict among those who have had experience with the serum seems to have been that the serum treatment produced a favorable rather than an unfavorable impression, which, after all, is scanty praise.

Jegunoff administered the serum intravenously, together with physiological salt solution, giving 140 c.c. of serum with 500 to 700 c.c. of saline to start with, and a second injection of 80 to 120 c.c. of serum within seven and one-half to twenty-three hours after the first.

TYPHOID FEVER

While antisera against typhoid fever have been proposed by a number of different observers, their value seems to be so problematical that their discussion may very well be omitted at this place.

PLAGUE

Against plague also antisera have been produced, which seem to be essentially of bactericidal nature (Yersin), though the preparation of Lustig may have feeble antitoxic properties. Both the serum of Lustig and that of Yersin have been tried out by the Plague Commission of India, but the reports are not very encouraging. Whether its use in combination with vaccination might not prove of greater value than vaccination alone, and especially in persons who have been actually exposed to the infection, future investigations will have to decide, but would, *a priori*, seem likely.

BACTERIOLYTIC-BACTERIOTROPIC IMMUNIZATION.

Among the bacteriolytic-bacteriotropic immune sera which find employment in the treatment of maladies to which the human being is subject, the most important are those which are directed against

infections with the pyogenic cocci, viz., the meningococcus, the streptococcus, the pneumococcus, the gonococcus, and the staphylococcus. Of these the antimeningococcus serum is, however, practically the only one with which notable curative results have been obtained. It will accordingly be considered in some detail, while the remainder need not occupy our attention to any great degree.

MENINGOCOCCUS MENINGITIS

Attempts to produce an antiserum for the treatment of meningococcus meningitis in the human being have notably been made by Flexner and Jobbling, Kolle and Wassermann, and Jochmann, and it may be said that the efforts of all these investigators have been crowned with a great degree of success. From the therapeutic standpoint very little difference indeed appears to exist in the efficacy of the three preparations in question, but there is still a good deal of difference of opinion in regard to their mode of action. All three contain agglutinins, precipitins, complement binding antibodies, bacteriolysins, bacteriotropins, and possibly also some antitoxins. From the different accounts that have been given, the conclusion suggests itself that while antitoxins may possibly play a role, this is unquestionably of minor importance when compared with the marked inhibitory effect which the serum exercises upon the multiplication of the organisms, and to its manifest bacteriotropic action, as evidenced by increased phagocytosis.

Flexner thus records that in two children who had received subdural injections of his serum, scarcely any extracellular diplococci could be found after the first treatment, while the number of intracellular cocci was much reduced, and that cultures could no longer be secured, even though the free forms had not yet disappeared altogether.

Flexner suggests that the phagocytic digestion not only prevents further multiplication of the diplococcus, but also that it detoxicates the endotoxin by reducing it to simpler and non-toxic or less toxic compounds.

That bacteriolysins *per se*, however, may also play a role is suggested by the observation that in a few instances in which the antiserum was injected into the spinal canal of monkeys infected

with the diplococcus the microorganisms disappeared without marked phagocytosis, though more slowly than in the cases in which outpouring of leukocytes was considerable.

Preparation of the Antimeningococcus Serum (according to Flexner and Jobling).—Horses are first injected subcutaneously with cultures of the diplococcus that have been heated for thirty minutes at 60° C., as many different strains being used collectively, as possible, so as to give rise to a polyvalent serum. As first dose the equivalent of a quarter surface test-tube growth on sheep-serum agar is recommended. At each subsequent injection the dose is doubled until an amount equal to four test-tube growths is given at intervals of five to seven days.

In the earlier work of Flexner and Jobling, intravenous inoculations were then substituted for the subcutaneous, beginning with one dose of living diplococci, the dose being progressively increased to two, three, five, etc., oeses, then to one-half, three-quarters, one, two, etc., agar slant cultures, and finally to one and a half bottles (12 oz. Blake) of surface growth. As the larger injections caused very severe reactions and alarming symptoms, they were discontinued, and subcutaneous and intravenous injections of autolysates¹ substituted, the dose being gradually increased from 1 to 3 c.c. and given about a week apart. Since the intravenous injections of the autolysates, however, likewise produced quite serious symptoms, they also were abandoned, and at present subcutaneous injections only are recommended for the whole process of immunization, living diplococci and autolysates being used alternately at intervals of a week. The maximum dose of living organisms and of the autolysates is one and one-half bottles.

The process of immunization in Flexner's horses was continued for a year or longer, before any of the serum was used for purposes of treatment.

Standardization.—Unfortunately no method is at present available by which the curative or protective effect of the antimeningococcus serum can be gauged other than by actual trial. Kolle and Wassermann attempted to standardize their serum on the basis of its content in complement binding antibodies, in the belief that these were identical with the bacteriolytic amboceptors. This idea,

¹ Meningococci which have been allowed to undergo self-digestion.

however, has been shown to be erroneous, and the method is from this standpoint therefore inapplicable. Other investigators have suggested to use the bactericidal power of the serum *in vivo* as indicator of its therapeutic properties. The values which can thus be obtained are, however, approximative at best, and the same is to be said regarding Neufeld's suggestion to gauge its strength by a determination of its bacteriotropic titer. Kraus and Doerr, in the belief that the efficiency of the serum depends upon its content of antitoxins, suggest its standardization upon this basis, in a manner analogous to the standardization of diphtheria antitoxin, but it is extremely doubtful whether these actually play an important role, and the suggestion has hence not met with favor. Under these circumstances it is apparent that the main stress must be placed upon the duration of the immunizing process, possibly coupled with a study of its bactericidal power *in vivo*, and its bacteriotropic effect.

Dosage and Mode of Administration.—From what has just been said, it is clear that the dosage of the serum still rests upon an empirical basis. As initial dose, Flexner recommends an injection of 30 c.c., which may be repeated every twenty-four hours for three or four days or longer. All injections should be made into the sub-arachnoid space, care being taken that the serum is introduced very slowly, so as to cause no symptoms of pressure. It is hence best to allow at least as much fluid to escape as it is desired to introduce.

As the best results are obtained in early cases (see below) every effort should be made to reach a definite diagnosis as soon as possible, and to this end spinal puncture is practically imperative. If this reveals a turbid fluid the antiserum may be injected at once, the microscopic and bacteriological examination being carried out later. If this should prove that the case was not one of meningococcus meningitis, no harm will have been done, while in the event of a confirmatory diagnosis, valuable time will have been gained. The appearance of the fluid at subsequent examinations, aside from the physical condition of the patient, will then be a fairly good index as to the necessity of repeating the injections. So long as this is cloudy further treatment is needed. All in all it is better to inject too much and too often than too little and too infrequently. Late in the disease, however, when chronic hydrocephalus has developed, the treatment is useless.

The subcutaneous or intravenous use of the serum is to be deprecated, as the results following this method of administration are no better than under the expectant plan of treatment.

One question of great practical importance which has arisen in connection with the serum treatment of meningococcus meningitis is whether any danger due to *anaphylaxis* is to be anticipated from the repeated injections, particularly since these are made into the sub-arachnoid space and since Besredka has shown that the direct injection of the alien serum into the central nervous system is particularly fatal to guinea-pigs. So far as we can tell, this danger is really a negligible quantity, especially as the daily injections in the early course of the treatment do not enter into consideration, and the patient usually is beyond the need of serum by the time that anaphylactic reactions would be expected to occur. But even in cases where the injections were continued into this period, serious symptoms have not been observed.

Results.—So far as the results of the serum treatment upon the course of the disease are concerned, we have sufficient evidence to show that through its introduction, one of the most fatal diseases, and one of the most dangerous in its late effects, even in cases where recovery has occurred, has lost some of its terrors. As regards its effect upon the mortality, much depends upon the time at which it is instituted. Of 241 cases which had thus been injected with the Flexner serum during the first three days of the malady, only 25.3 per cent. died, while a delay of from one to four days beyond this period increased the death rate to 27.8 per cent., and a still further delay to 42.1 per cent. The general death-rate of 712 treated cases was 31.4 per cent., as contrasted with the usual mortality of from 53 to 90 per cent. By eliminating all those cases where the patients were first seen in an already hopeless condition, but injected nevertheless, Flexner calculated an average mortality of 25.4 per cent. Similar results have been reached with the sera prepared by Wassermann, Jochmann, and Dopter. The latter claims an average mortality of only 16.47 per cent. (402 cases) for his serum, as contrasted with one of 65 per cent. in untreated cases; Schöne one of 27 per cent. for Jochmann's serum (in a relatively small number of cases), and Dopter one of 18.35 per cent. (158 cases) for that of Wassermann.

The immediate effect upon the malady is also quite favorable;

usually within twenty-four to forty-eight hours there is definite improvement, as evidenced by a return to consciousness, disappearance of delirium, diminution of the general *hypersensibility*, etc. The duration of the disease is shortened to eight to twelve days, as contrasted with five weeks or longer, which is the rule in fully one-half of the cases that end in recovery, in the absence of serum treatment.

In conclusion it would seem that late effects of the malady are only exceptionally observed; mental disorder, paralysis and blindness in particular are only rarely seen.

We may accordingly look with pride and satisfaction upon the antimentingitis work as one of the brightest pages in the history of serology.

STREPTOCOCCUS INFECTIONS

Since the days when v. Behring first came forward with the announcement that it is possible with the serum of an animal that has been immunized against the corresponding toxin, not only to protect individuals against diphtheria, but even to cure the disease after this has once developed, attempt after attempt has been made to produce an effective antiserum also against streptococcus infections. But as yet the problem has not been solved. Much work of value has been accomplished, but still more remains to be done. That it is possible to protect animals against a fatal infection with streptococci by means of a corresponding antiserum had been shown by v. Behring himself in 1892, and shortly after a number of French observers attempted to influence the infection in the human being also in a similar manner.

The most noteworthy of these early attempts is intimately connected with the name of Marmoreck. This investigator, believing in the unity of practically all the different types of streptococci which are pathogenic for man, succeeded in increasing the virulence of an angina strain by animal passage to such a degree that 0.000000001 c.c. was sufficient to kill a rabbit with acute symptoms. With this strain he immunized horses and sheep and then recommended the resulting antiserum, which is thus a monovalent serum, for the treatment of all forms of streptococcus infections occurring in the human being. The results, however, were practically *nil*.

If we come to investigate the reasons which may be responsible for this want of action, different possibilities suggest themselves. On the one hand it is conceivable that the identity of the different strains is only apparent and that Marmoreck's serum was inactive merely because it was *monovalent*, *i. e.*, because it had been produced with but a single strain. If this were so it would evidently be necessary to prepare a *polyvalent* antiserum, *i. e.*, to immunize animals with as many different strains as possible, and to use the resultant product. Or, one might imagine that in consequence of animal passage, to increase the virulence of a different strain, the organism could become so altered in its biological properties that its virulence for the human being would be diminished or lost, and that the corresponding antiserum, though active for the animals through which the passage had been conducted, might still be inactive in the human being. In such an event, animal passage would have to be omitted, and a monovalent or polyvalent serum prepared by immunizing directly with strains that had been obtained from human beings (*sc.*, with cultures made from human sources only).

Both possibilities have indeed been considered and practically tested. Denys and Van der Velde thus prepared a polyvalent serum from a number of different strains, whose virulence had been further increased by animal passage, but this serum also has fallen into oblivion, which suggests that subsequent investigations did not support the favorable reports which first followed its introduction. Tavel, Krumbein, and Paltauf, on the other hand, prepared polyvalent sera from different human strains without animal passage, and Menzer and Moser monovalent strains which had likewise not been passed through animals; while Aronson attempted a combined procedure making use of passed and unpassed organisms conjointly, both in the form of monovalent and polyvalent preparations. At the present time practically all these products are in use, and while they are unquestionably efficacious in the animal experiment, the clinical evidence is still rather against than in favor of their real value. This suggests the possibility, of course, that clinicians may not apply the sera as promptly in streptococcus infections as is done in diphtheria, and as a matter of fact there is a good deal of truth in this criticism. That this factor may actually be one of moment is suggested by the fact that the best results have thus far been obtained in scarlatina, where the diagnosis is reached at an

early date, and where the serum can be conveniently and systematically tested. In the other streptococcus infections the bacteriological diagnosis is frequently not made at all, or it is delayed until it would seem unreasonable to expect any favorable result. Here, as elsewhere, in serum therapy, the clinician should bear in mind that the greatest good will only be accomplished, if the various antisera are used early, in sufficient quantity, and usually in repeated doses.

Mode of Action.—Regarding the mode of action of the various antistreptococcus sera, it would seem that this is to a great extent bacteriotropic in character, for whereas in unprotected animals an intraperitoneal inoculation with an appropriate number of organisms is followed by a relatively insignificant hyperleukocytosis and phagocytosis, while the organisms multiply without any very evident restraint, the treated animals show exactly the opposite picture, *i. e.*, extensive hyperleukocytosis and phagocytosis without evidence of multiplication. The same can be shown outside of the body, directly under the microscope; for whereas in the presence of normal serum, washed leukocytes will scarcely take up any virulent streptococci, they do so readily when in contact with immune serum.

Whether or not bacteriolytic processes also play a role in the protection of the animal with suitable immune sera is still a matter of dispute. Antitoxins, on the other hand, certainly are not present.

Preparation and Standardization.—The preparation of the antistreptococcus sera is conducted essentially on the same lines as that of other non-antitoxic sera, *viz.*, by starting the immunization with small doses of killed-off cultures and progressively increasing the dose, until finally full virulent living organisms are injected. At the Serum Institute of Vienna, bouillon cultures of from two to eight days' growth are used, the initial dose being 0.5 c.c., and the final one varying between 100 and 200 c.c., all the injections being given subcutaneously. The animals are not bled until the immunization has been continued for about six months. The serum is then tested in reference to its bacteriological purity and titer, and finally put up in doses of 50 and 100 c.c. each, without any preservative.

In making up the polyvalent antigen for immunization, it is convenient, even though all the other strains be of human origin and not passed through animals, to introduce one strain which has been so treated and brought to a high degree of virulence, for mice for

example, so as to have an approximative indicator at least for the potency of the antiserum, this being then standardized against that particular "animalized" strain. At the same institute that dose of streptococci which will kill a white mouse at the expiration of or just preceding the end of four days is designated as a single lethal dose; but in testing an antiserum, ten times this amount is chosen as the dose against which one unit of antiserum should afford protection. A simple normal serum is one of which 0.01 c.c. will afford this degree of protection, and 1 c.c. of such a serum is said to contain a single immunization unit, and 1 c.c. will accordingly protect 1000 mice against a single lethal dose each. The Vienna serum, as it is now marketed, contains 20 to 40 units to the cubic centimeter.

Dosage and Uses.—*Prophylactic Doses.*—For prophylactic purposes, antistreptococcus serum has been recommended in connection with scarlatina and the puerperal process, either by itself or in combination with the use of a vaccine. To prepare the latter, F. Meyer suggests that a bouillon culture of a corresponding strain be obtained, centrifugalized, the sediment washed repeatedly with saline, and finally emulsified with a quantity of 0.5 per cent. carbolic acid in saline, equal to the volume of the initial culture. The resultant emulsion is killed off by heating for six hours at a temperature of 65° C., when it is tested bacteriologically and shaken overnight in a shaking machine. This constitutes the finished vaccine, which does not need to be counted out. The individual in question receives a serum injection of 20 c.c., and at intervals of three days increasing doses of the vaccine (0.1, 0.2, 0.4, 0.8, and 1.6 c.c.).

Curative Dose.—For curative purposes the serum has been used in scarlatina, in severe streptococcus infections of the throat, in erysipelas, in puerperal streptococcus infections, in chronic streptococcus infections associated with tuberculosis and malignant growths, in streptococcus endocarditis and arthritis, etc. In scarlatina the treatment is indicated especially in those cases in which the throat infection is at all severe, or in which the initial general symptoms suggest the likelihood of a severe course. In cases of the first type the injection of 50 to 100 c.c., given subcutaneously, and repeated once or twice, if necessary, is usually sufficient, while in severe systemic infections, when the blood examination frequently shows the presence of large numbers of organisms, still larger doses, and repeated even more frequently, are advocated. In cases of pro-

tracted sepsis, vaccination (see above) may well be combined with the serum treatment. In fulminating cases, where blood examination reveals the presence of streptococci already within a few hours of the first appearance of symptoms, nothing short of an intravenous injection (50 c.c.) should be tried, and it would seem worth while in just such cases, in fact in all the more severe infections, to inject the serum diluted with normal salt solution, as has been suggested by F. Meyer, or to follow its injection with a subcutaneous infusion of 500 c.c. or more.

In severe streptococcus anginas the dosage is essentially the same *i. e.*, 50 c.c., given subcutaneously and diluted, if desired, the dose being repeated in accordance with the urgency of the symptoms, and two injections a day given if necessary.

In erysipelas the use of the serum is advocated especially in cases affecting the head and neck, as also in migratory cases, while the facial type of the disease usually does well with ordinary treatment. The dosage here also ranges between 50 and 100 c.c. according to the gravity of the case.

In puerperal infections the rule should be to use the serum early or not at all. A great deal of valuable time is here often lost in waiting to ascertain whether the infection will not cure itself. The patient should receive the benefit of the doubt, no matter whether the statistics are thereby unduly turned in favor of the serum or not. Its use is logical and should be resorted to in every case where fever develops during the puerperal period, if this is not manifestly sapremic in character. 50 c.c. given subcutaneously is sufficient in the milder cases, while in the presence of ominous symptoms larger doses should be employed (100 to 200 c.c.), which here, also, may be suitably combined with a subcutaneous infusion of saline (500 to 1000 c.c.). In urgent cases intravenous injections should be made (50 c.c.). After hysterectomy it is recommended to give an intraperitoneal infusion of 500 c.c. of serum with 1000 c.c. of saline, the operation being preceded by an intravenous injection of 100 c.c. In cases which have become chronic the serum treatment should be combined with the use of an autogenous streptococcus vaccine.

In the chronic infections associated with endocarditis, arthritis, tuberculosis, and carcinoma, etc., much smaller doses are given, *viz.*, 5 to 20 c.c., as larger amounts are apt to cause an aggravation of

some of the symptoms, and notably temperature disturbances lasting for sixteen to twenty-four hours. But in these cases more good may, *cæteris paribus*, be expected from the use of a vaccine which should, if possible, be autogenous, than from the serum. (Both may, however, be advantageously combined.)

Results.—Upon surveying the literature in reference to the curative value of antistreptococcus serum, one is struck with the fact that while diphtheria antitoxin is generally used as early as possible, the antistreptococcus serum is usually resorted to too late and in insufficient amount. The result is, that from a statistical standpoint the general verdict has been rather unfavorable. This emphasizes the importance that immunization treatment in hospital work particularly should be placed in the hands of especially trained men, who should be consulted in doubtful cases. My belief is that then and only then immunotherapy will yield its best results.

As I have already indicated, the most favorable reports have been published in connection with the use of the serum in scarlatina. Escherich, speaking of the effect of the Moser serum, remarks that this is "zauberhaft" (magic), and especially so when used early. Of 112 cases which had been injected on the second or third day every one recovered, while among those in whom the treatment had been delayed the mortality ranged between 13 and 50 per cent.

In erysipelas, very curiously, the least favorable results have been obtained; in the migratory forms, however, the disease usually comes to a standstill in from three to four days. The facial cases, of course, should not be included in an analysis of the results, as they usually do well without serum treatment. In puerperal cases the testimony is most conflicting. Some observers, such as Bumm, Peham, and Burkard, thus speak quite favorably of its use (when employed early), Burkard reporting 50 cases, of which twenty-nine were pure streptococcus infections, without a single death, while others deny having seen any good accomplished whatever. It is in these very cases that I would advocate that the serum treatment should be placed in the hands of experts who shall decide how the serum is to be given, when it is to be given, and how much is to be given. That even then there will be unfavorable results also is to be expected, but it would stand to reason that the maximum amount of good that *could* be accomplished would be obtained under such conditions.

Regarding the value of the serum in other streptococcus infections, too little is as yet known to warrant any definite conclusions. Here also is a large field for the expert, and until it is tilled by him the results can hardly be expected to be what they should be. As I have suggested, the best results may here be expected from serum treatment and vaccine treatment conjointly.

PNEUMOCOCCUS INFECTIONS

While the results which have been hitherto obtained in the serum treatment of pneumococcus pneumonia have been rather disappointing, Neufeld and Händel have recently pointed out that this may have been due in part to the administration of sera of too low a titer and of insufficient amounts, in part to the administration by the subcutaneous route, and in part to the use of sera which did not correspond to the same strain as the infecting organism. The same observers could show that the so-called polyvalent sera of commerce were in reality not truly polyvalent, and possessed protective value only against a certain group of pneumococcus strains, while they were of no avail against other types. They emphasize that no curative properties can be expected from a given serum unless this is homologous for the type that is causing the infection. It will accordingly be necessary to resume the serum treatment of pneumonia from this standpoint, and to establish the type of the organism in every case before an ultimate verdict upon the subject can be reached.

A serum which possesses a high degree of protective as well as curative value against the common strains of pneumococci, in the animal experiment, is at present prepared under Neufeld's direction by the Serum Institute of Saxony, and may be recommended for use in infections with the corresponding strains. Its titer is such that 1 c.c. will protect 5000 grams of body weight (tested against white mice weighing from 18 to 20 grams) against 0.1 to 0.0001 c.c. of a pneumococcus bouillon culture, the fatal dose being 0.000001 or less. Neufeld and Händel point out that it is essential to test out the serum against large or medium doses of the organism as the values obtained in the case of small doses do not apply to corresponding multiples. Of a serum, for example which would protect in a

dose of 1 c.c. against the minimal fatal dose, 5 c.c. would not necessarily protect against five multiples of the fatal dose and so on. They ascertained that there is a certain threshold of action beyond which a given dose will protect against many multiples (up to a million) of fatal doses, while below this point the activity of the serum rapidly diminishes and may indeed be *nil*.

In a concrete case the question naturally arises whether the type of the infection corresponds to the strains with which the serum was produced. To this end it is recommended to inject a mouse with a small quantity (0.5 c.c.) of the patient's sputum to which one titer dose of the serum has been added. If then the animal does not succumb within the next twenty-four hours we may assume that the serum is homologous to the type of the infection and would hence be applicable.

Mode of Action.—As regards the mode of action of his antipneumococcus serum Neufeld lays special stress upon its bacteriotropic effect. It is possible, however, that it may have a certain bacteriolytic as well as antitoxic component as well.

Dosage and Mode of Administration.—As the absorption from the subcutaneous tissue is notoriously slow the serum should be injected intravenously whenever possible, or intramuscularly, if for any reason (as in young children) the other route is excluded. The initial dose for an adult is 40 to 50 c.c., and for children one-half of this quantity, and may be repeated on the following day. If the patient has been previously treated with horse serum it is recommended to inject a small quantity (about 0.5 c.c.) a few hours before the principal injection is given, the idea being to guard against anaphylactic reactions by the production of the antianaphylactic state. Following the second injection no anaphylactic disturbances need of course be anticipated (for the same reason). Above all it is essential to inject as soon as the diagnosis has been made.

Results.—Regarding the results which may be obtained with antipneumococcic sera prepared along the lines which have been indicated by Neufeld and Händel, it would be premature to make any definite statements. Suffice it to say that the treatment has no appreciable influence upon the pathological-anatomical condition of the lung, but that the general condition of the patient is apparently improved and that an early crisis may be expected if the serum is administered early. To what extent the death-rate will be affected

by the treatment is impossible to foretell from the meager data which are now available. Of the thirty-seven cases treated by Betz and Géronne, five died, but of these several were tubercular, and one in an extremely critical condition, when injected (on the sixth day).

In *localized infections* with the pneumococcus it would seem advisable to combine the serum treatment with the use of sodium oleate and boracic acid, as recommended by Lamar (see section on Chemotherapy).

STAPHYLOCOCCUS INFECTIONS

While several attempts have been made to combat staphylococcus infections with corresponding antisera, we know too little as yet of their mode of action and their effect as to warrant more than a mere statement of this fact. Noteworthy clinical results have apparently not as yet been achieved. The great question here as elsewhere in the treatment of bacterial infections with antisera is whether or not all the organisms are of one type. If this should not be the case it is clear that only a homologous serum would likely be of value, and as a matter of fact, evidence is rapidly accumulating which goes to show that marked differences actually exist between staphylococci derived from different sources.

ANTIGONOCOCCUS SERUM

Of late an antigonococcus serum also has been placed upon the market, for which good results have been claimed. *Torrey's serum* is prepared by immunizing sheep with gradually increasing doses of dead, and later, of living cultures of virulent strains, and is marketed in 2 c.c. ampoules, which amount represents a single dose. Repeated injections are made at intervals of one, two, three, or four days, according to the requirements of the individual case. Its use is advocated in chronic conditions produced by gonococcic infection, as in those arising from a direct extension of the primary infection into organs like the prostate, epididymis, testicles, bladder, and Fallopian tubes, as also in cases of gonococcus arthritis, iritis, endo-

carditis, pleuritis, and meningitis. As yet not enough is known of the effect of the injection upon the maladies in question to warrant any definite statements.

In the booklet on the subject which has been issued by the manufacturers the statement is made that within a year 10,000 doses of the serum had been sent out for experimental purposes, and that of the cases reported upon 58 per cent. showed decided benefit, and that in 17 per cent. only the results had not been favorable. Future investigations here also are needed to establish the actual status of the treatment, which, *a priori*, of course, would seem logical, and especially so when combined with corresponding vaccination.

AUTOSERUM THERAPY

A number of investigators have suggested the administration of the patient's own serum under various pathological conditions upon grounds, it must be admitted, which in some cases seem rather lacking in force. Gilbert and Fede thus recommend the subcutaneous injection of small quantities (1 to 2 c.c.) of the patient's own exudate in the treatment of tubercular peritonitis and pleurisy, and claim that the absorption of the exudate is thus greatly hastened. Similar results have been reported by Senator and Schnütgen. Modinos reports that the injection of moderate doses (about 8 c.c.) of the patient's own serum is of beneficial influence upon the course of various acute infectious diseases, and cites specific cases of influenza, typhoid, and Malta fever. Others have reported favorable results from the injection of small doses (1 to 2 c.c.) of serum in gonorrheal arthritis, and so on.

A few years ago Hodenpyle met with an instance of carcinomatosis associated with chylous ascites in which the disease seemed to have been arrested, and in which he assumed that protective antibodies (cytotoxins) were present in the circulation. He used this fluid in large quantities in a number of cases of cancer, and for a time apparently with a beneficial effect. The results, however, were not lasting, and so far as my knowledge goes none of the treated patients are now living. The donor, moreover, died within a year of the time the treatment of the patients was begun. Following these experiments a number of investigators used the ascitic fluid

of cancer cases in the treatment of the corresponding patients, but likewise without any lasting benefit.

Autoserum Therapy in Syphilis of the Central Nervous System.—Of late the patient's own serum has been advocated in the treatment of syphilitic diseases of the central nervous system, the serum being administered by the subdural route, and obtained from the patient one hour after an intravenous injection of salvarsan. The underlying idea is to bring both the salvarsan and any antibodies that may be formed in the patient's own body into as intimate a contact as possible, with the spirochetes, located in the perivascular lymph spaces and the adventitia of the bloodvessels. This plan seems thoroughly logical and deserves extended investigation. That protective antibodies are actually present in the blood serum of treated syphilitic patients has been demonstrated beyond a reasonable doubt. It has thus been observed by Taege that the milk of syphilitic mothers, after treatment with salvarsan, develops curative properties for the infected infants, and other investigators have noted marked improvement in adults following the injection of the serum of treated syphilitic individuals. The effects, however, were not lasting, and it hence appears rational to combine the use of the salvarsan with that of the serum. The salvarsan, moreover, when given by itself, by the subdural route is too irritating to warrant its use in this manner, while in combination with serum this objectionable effect apparently falls away.

Technique.—The procedure which has been advocated by Swift and Ellis is the following: One hour after an injection of salvarsan or neosalvarsan, 50 c.c. of blood are withdrawn from one of the veins at the bend of the elbow, to which end a MacRae puncture needle will be found most convenient, the blood being aspirated directly into large tubes. The serum is allowed to separate out overnight (in the ice-box), and the following morning is diluted to 40 per cent. with normal saline. At that time approximately 15 c.c. of spinal fluid are withdrawn and replaced by 30 c.c. of the diluted serum, which has previously been heated for one-half hour at 56° C. and then brought to the temperature of the body. The patients are kept in bed for twenty-four hours, the foot end being raised for about one hour following the treatment.

After two or three weeks the injection is repeated and so on, according to the symptoms of the individual case. As a rule there

is but little reaction after the injection, except in tabetics in whom lightning pains may occur, or become more violent for a while, if they previously existed.

Results.—Too little time has elapsed since this method of treatment was first advocated, and too small a number of patients has as yet been treated to warrant any far-going conclusions.

Swift and Ellis mention four tabetics, in whom the cell count in the cerebrospinal fluid promptly fell to normal, while the globulins decreased in amount much more rapidly than during the previous treatment with salvarsan and mercury alone; at the same time the Wassermann reaction in the spinal fluid became negative in two of the patients, even when 0.5 c.c. of fluid was used in the test. In two other patients, however, the treatment had little effect on the Wassermann.

As the writers suggest, future experience may show that the best results may be obtained by further enforcing the beneficial effect of the serum by the addition of neosalvarsan. They mention that in one instance the patient received 0.5 milligram of the neosalvarsan diluted with 12 c.c. of normal serum plus 18 c.c. of normal saline, as a first injection, which was followed ten days later by one of 1 milligram, similarly diluted, without causing any undue reaction.

NORMAL SERUM THERAPY

Under various pathological conditions *normal* serum also has been injected for curative purposes and favorable results have been observed in many instances. A number of investigators thus report a remarkable influence upon certain toxicoses of pregnancy. Mayer mentions several cases of various types of dermatoses (herpes, urticaria, pruritus) besides a case of eclampsia, and one of acroparesthesia of the finger tips, in which excellent results followed the injection of serum from a normal case of pregnancy. He concluded that the serum of the patients in question was deficient in normal protective substances against the poisons which are absorbed from the developing embryo, and that these substances are specific in their nature. Freund, on the other hand, while confirming the beneficial effects of the serum of normally pregnant women upon the toxicoses referred to, obtained equally satisfactory

results with normal serum from non-pregnant women, as also from men, horses, etc. Corresponding observations have since been published by others.

Favorable results have also been observed by many investigators, following the injection of normal serum or of defibrinated blood, in various hemorrhagic conditions. Weil thus found that the subcutaneous injection of 30 c.c., or the intravenous injection of 15 c.c. of fresh human or animal serum has a markedly beneficial effect upon the bleeding of hemophiliacs. Leary reports several cases in which following a preliminary injection of 30 c.c. of normal rabbit serum operations could be carried on without subsequent bleeding of note. Welch cites twelve cases of hemophilia neonatorum, all of which recovered after the subcutaneous injection of fresh human serum (average, 80 c.c. in 10 c.c. doses, distributed over four days).

Other observers obtained favorable results in cases of rheumatic purpura with intestinal hemorrhages, in uterine bleeding, in intestinal bleeding in connection with cirrhosis of the liver and typhoid fever, in hemorrhagic retinitis, etc. Moss suggests that in those cases in which a notable degree of anemia has already developed, the injection of large quantities of defibrinated human blood is probably the best procedure, care being taken that a donor is selected whose serum will not agglutinate or hemolyze the red corpuscles of the recipient. In other cases normal rabbit serum will be found to be just as efficacious. It has the advantage, moreover, of being obtainable without difficulty, of being very little toxic, while the chances that the patient has been rendered hypersensitive by previous injections of rabbit serum are rather remote.

While human blood has also been used quite extensively in the treatment of advanced cases of pernicious anemia, leukemia, splenic anemia and pseudoleukemia, lasting effects have not been achieved, while the immediate results, owing no doubt to the introduction of the large number of normal red cells, may be quite satisfactory.

CHAPTER XIV

CHEMOTHERAPY

IN the foregoing chapters we have seen that the animal body has at its disposal a mechanism by means of which it is not only capable in many instances of preventing infection, but even of overcoming this successfully if by any chance microorganisms have once passed the outer barriers and have gained a foothold in the tissues proper. We have also seen that it is possible to introduce some of those substances which the body makes use of in its defence, from without, and that we can frequently turn the balance of the scales toward recovery in this manner, where unaided this would have been impossible, or attended by grave danger. Nevertheless we must admit that only too often all our efforts to combat infection by the body's own methods are in vain, and that in the majority of infections we are still far from a successful treatment.

In view of the fact that in the test-tube we are able to destroy microorganisms with the greatest ease, by the aid of a large number of chemical preparations, the thought has naturally suggested itself whether it would not be possible to assist the normal defences of the body by the administration of some of these substances. We know as a matter of fact that the only specific medicinal treatment of the older Pharmacopœa, viz., that of malaria by means of quinine, and of syphilis by means of mercury, depends upon the destructive effect of the remedies in question upon the respective parasites. The recognition of this fact is of recent date, however, and does not form the basis upon which the treatment of these diseases was established. The discovery of the therapeutic properties of quinine and mercury, in other words, was not the outcome of logical thought and corresponding experimentation, but purely accidental.

But the fact that it is actually possible to destroy some of the pathogenic microorganisms in the body of an infected individual by chemical means, would suggest that a similarly fortunate result might be achieved with other substances in the case of other organ-

isms. The earlier investigations in this direction were, however, not crowned with success, and it was soon realized that in these studies also, accident would probably have to play a role, unless indeed every chemical substance were individually tested. The first and most formidable difficulty which was encountered depended upon the fact that the majority of those substances which have strong germicidal properties, when tested outside of the animal body, were promptly rendered innocuous by entering into chemical combination with the albumins of the blood, when introduced into the body, and if a certain dose was exceeded their toxic effect was such that any attempt at destruction of the parasites would have carried with it the destruction of the host. I well recall an interesting observation which illustrates this point. A patient suffering from pneumonia was accidentally given a dose of bichloride of mercury which nearly caused the individual's death. He was saved with difficulty, but died of his pneumonia a few days later. Evidently the dose of the bichloride, though large enough to have nearly killed the patient, had not been sufficiently large to kill the offending parasites.

Organotropism and Parasitotropism.—In work of this nature the distribution of the poison in the body is evidently of prime importance. If, aside from any binding action on the part of the circulating albumins, the affinity of the poison is greater for the tissues of the body than for the protoplasm of the parasites, or to use the parlance of Ehrlich, if the poison is more markedly organotropic than parasitotropic, it is evidently not suitable for therapeutic purposes, and especially so, if at the same time the toxic dose for the macroörganism should be smaller than that for the microörganism. The great problem then has been to discover substances which, while possessed of germicidal properties, or what amounts to the same thing, of the power to inhibit reproduction of the parasites, shall also be non-toxic, or but little toxic for the macroörganism, and more markedly parasitotropic than organotropic.

In this investigation, Ehrlich, to whom we are already indebted for so much of our knowledge of the more intricate problems of cell life, has again taken the leading position, and may indeed very appropriately be styled the father of modern pharmacology and chemotherapy. Since the degree of antibody formation in systemic infections with protozoan parasites, in contradistinction to bacterial

infections, is usually insufficient in itself to successfully combat the corresponding maladies, the attention of Ehrlich and his collaborators, and many other noted investigators, has within recent years been largely centred upon these very infections. As a result of the study of several thousand different products in regard to their influence upon trypanosomes which are especially convenient test objects in this respect, it has been found that there are after all very few which can effect a cure in animals that have been infected with the parasite in question, but these are well characterized chemically, and belong to three distinct groups. The first of these comprises certain arsenical preparations, notably arsenious acid, atoxyl (arsanil), arsacetin, arsenophenyl glycin, and the dichlorhydrate of dioxydiaminoarsenobenzol (popularly known as preparation No. 606), and in addition to these certain antimony preparations. The second group is represented by certain azo dyes, such as trypan-red, trypan-blue, and trypan-violet, while certain basic triphenylmethane dyes, such as parafuchsin, methyl violet, pyronin, and others, belong to the third order.

Chemoreceptors.—The study of these products in their behavior to trypanosomes has led to a number of interesting discoveries. While Ehrlich originally assumed that the so-called side chains of the protoplasmic molecule only served purposes of nutrition, in other words, that all receptors were essentially *nutriceptors*, and that medicinal agents were not bound in this manner, he now holds that receptors do exist by which such substances may be bound, and terms these *chemoreceptors*.

He suggests that the groups of the latter order in accordance with their simpler functions are probably of a less complex structure, that they are more firmly attached to the cell, and are hence less readily cast off, and that as a consequence of their "sessile" character, crystalline chemical substances are, generally speaking, incapable of eliciting the liberation of corresponding antibodies. This conclusion was based upon the following observation:

If a given strain of trypanosomes is continuously treated with chemotherapeutic agents belonging to one of the groups referred to above, a race of organisms develops which can no longer be influenced by that particular product and which is accordingly said to be "fast," in reference to that particular drug. It is interesting to note that this acquired resistance or "fastness" is in a large measure specific.

A strain which has been rendered resistant to trypan-red and which is also fast to trypan-blue and violet is thus non-resistant to arsenic and the triphenylmethane dyes, while one which has been rendered arsenic-fast is resistant only to this and not to the trypan-dyes and the triphenylmethanes, etc. This fastness, it was then ascertained, remained a constant character through innumerable generations, so long in fact as the organism multiplies by direct division, while it is lost in the descendants of sexual reproduction.

The attempt to explain the development of such drug-fast strains, as I have just said, led Ehrlich to assume the existence of chemoreceptors. Since arsanil itself is non-toxic, while its reduction products are capable of killing trypanosomes in high dilution, it follows that the trivalent arsenical radicle which is in combination with the benzoyl radicle must in some manner be anchored to the trypanosomes; and as the toxic effect of the arsanil is lost in the so-called arsenic fast strains, the conclusion suggests itself that the untreated parasite must possess a definite group or receptor with which the arsenical group can unite, and which is capable of undergoing a certain modification, in consequence of which its affinity for the preparation in question is lost, or at least diminished. In the absence of such a combining group it would be very difficult to explain why the treated strain should be arsenic resistant and the non-treated arsenic susceptible.

Drug "Fastness."—In a former chapter we have seen that a certain type of immunity results from the occurrence of receptoric atrophy,* and Ehrlich has shown that during the process of serum immunization, trypanosomes may develop a serum fastness which is of this character; where, in other words, those nutriceptors of the parasite, which are continuously occupied by a corresponding antibody furnished by the host (the rat for example), disappear or are replaced by receptors of a different structure, through which the nutrition of the cell can again be carried on. In studying the nature of drug fastness, Ehrlich then ascertained that this is not dependent on atrophy of the corresponding receptors, but upon a modification in their structure, as is evidenced by the fact that by changing the structure of the *arsenical* product, for example, this may still be forced upon the parasite, so to speak, and lead to its destruction.

The recognition of this possibility is, of course, of the greatest importance, as it shows the lines along which such parasiticidal

substances must be constructed, in order to produce the maximum amount of effect with the least chance of leading to the development of an insurmountable drug resistance. That this can be done, Ehrlich himself has demonstrated in a perfectly satisfactory manner. He could thus show that mice which had been infected with arsanil-fast trypanosomes could be cured with arsenophenyl glycin, even at a time when death seemed to be imminent. The problem will, of course, be the more difficult the larger the number of drug-fast strains that is possible, and not only this, but the larger the number of serum-fast strains that may develop. For we must bear in mind that the destruction of the parasites in question is of necessity followed by the development of corresponding antibodies, which in itself is, of course, a favorable occurrence.

If, however, the destruction of the trypanosomes by the arsenical preparation, for example, has not been complete, and if the resultant antibodies do not succeed in killing off the rest, there is a strong probability that a serum-fast strain will now develop and bring about a relapse (relapse strain No. I). When some of these organisms then die or are killed by a repetition of the dose of arsenic, if indeed the strain is still susceptible to the same preparation, a new type of antibody will be formed which will be specifically directed against relapse strain No. I, from which a new serum-fast strain may then develop and cause a second relapse (relapse strain No. II), and so on, the number of serum-fast strains being limited only by the ability of the parasite to produce new receptors with which it can attend to its nutrition.

This implies, of course, that as the number of different kinds of foodstuffs which the parasite can utilize, progressively diminishes, a time will finally come when the infection will become eradicated spontaneously. This might occur relatively early, so that the infected animal or patient would actually receive the benefit of this fractional destruction of the parasites, but, on the other hand, there is a possibility that during each relapse vital structures may be damaged to such an extent that the individual would not live long enough to reach the point where the infection would at last have exhausted itself.

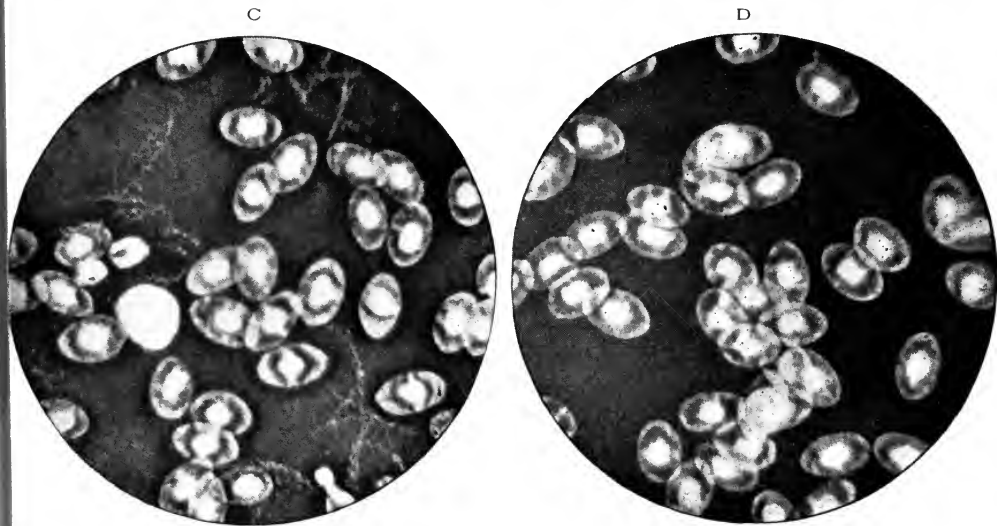
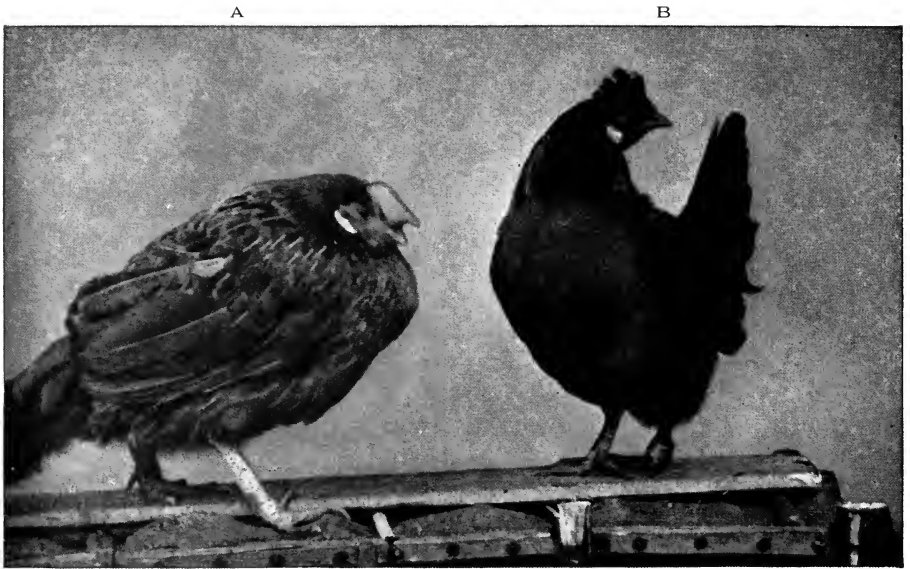
Examples in point are relapsing fever, on the one hand, and syphilis and sleeping sickness, and possibly also malaria, on the other. In relapsing fever we thus have evidence that only three or four different

serum-fast strains can exist, and we accordingly find that after a patient has safely passed through the two or three corresponding relapses, spontaneous recovery occurs, there being then antibodies present against the only strains that are possible in that particular *milieu*. In syphilis it is quite different. Here the number of food-stuffs that the spirochete can utilize is evidently quite large. In the untreated individual, relapse follows relapse, and the damage done to vital parts is only too often so extensive, relatively early in the course of the infection, that the patient succumbs, owing to the resultant injury, long before the disease has "worn itself out."

The discovery of this element of "fastness" or resistance on the part of microorganisms is evidently of the greatest importance, as it throws light upon many phenomena, the cause of which has heretofore been most obscure. The question has thus long remained unanswered, why the syphilitic individual cannot be re-inoculated with syphilitic virus while his disease is active. The reason now is quite evident. For we know that the spirochetal strains which at any time are operative in the body of the syphilitic patient are "fast" strains, of varying degree, and that antibodies are present in his blood which are specifically "tuned" to those of a lower order, *i. e.*, to those serum strains from which the "highest" ones have become developed, so that the "street spirochete," so to speak, if introduced under such conditions, must of necessity meet with those antibodies which would lead to their destruction. If once it were possible to cultivate all these different strains, then it would also be possible to reinfect the syphilitic individual, not with the street virus to be sure, but with a strain of a higher order of serum fastness, than would correspond to the number of antibodies that have already been formed. In the animal experiment, using trypanosomes, this can indeed be done at the present time, and at the Speyer Haus, in Frankfurt, Ehrlich has under cultivation all five of the serum-fast strains which are possible in the organism of the mouse.

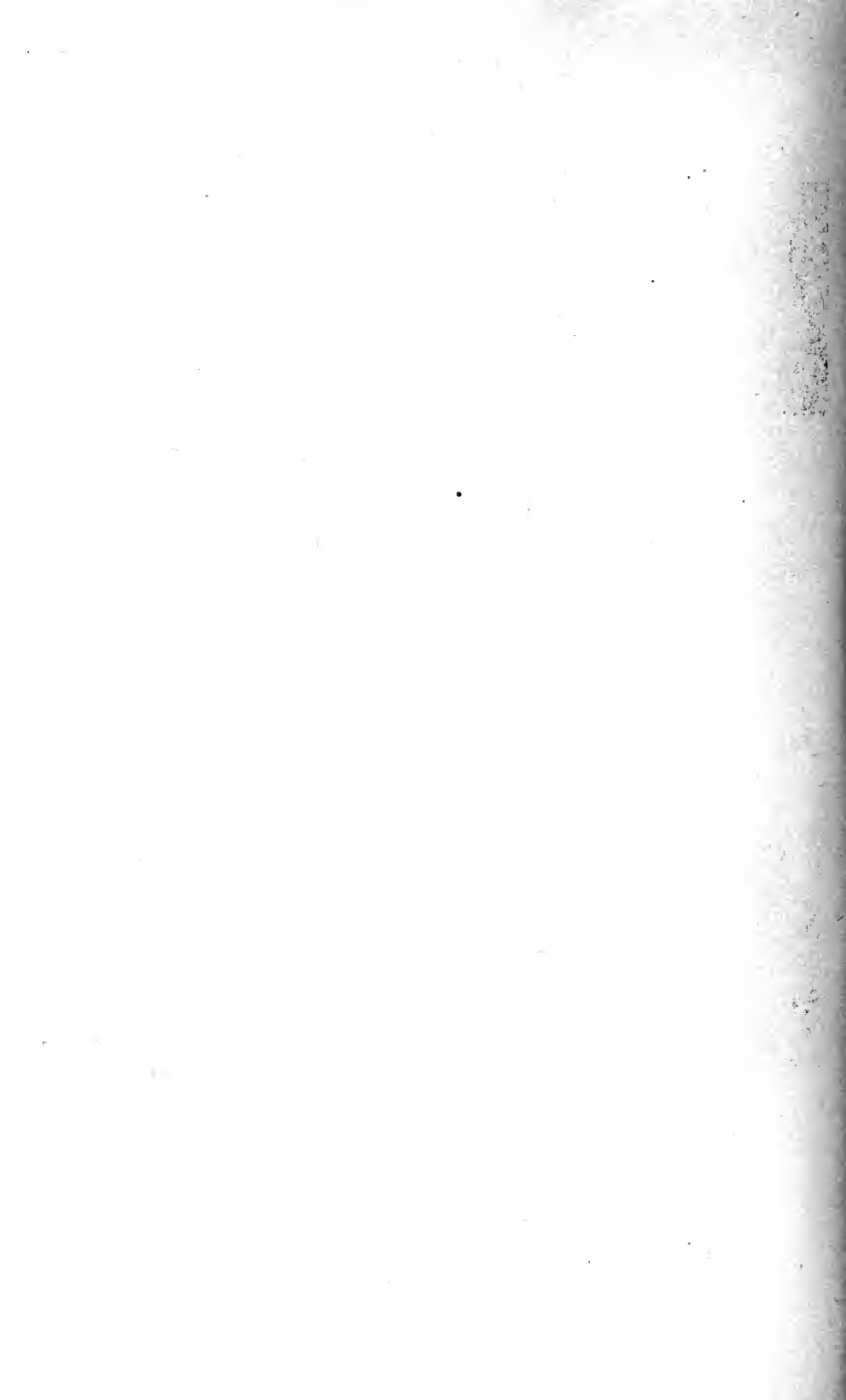
Therapia Magna Sterilisans.—As the development of "fast" strains is thus one of the greatest impediments to the successful treatment of the maladies in question, our efforts should be directed to the discovery of medicinal substances which should be capable of effecting the complete sterilization of the individual at one time (Ehrlich's *therapia magna sterilisans*). The demonstration that this is actually possible, not only in the infected animal, but also in the infected human being, we also owe to the indefatigable genius of Ehrlich.

PLATE IV



Showing the Effect of Salvarsan Treatment upon Spirillosis of Chickens. (Taken from Ehrlich and Hata.)

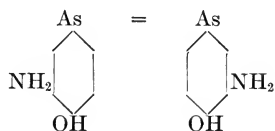
A, four days after infection, untreated; B, two days after infection, treated; C, control showing spirilla; D, a treated chicken showing absence of spirilla.



To give an idea of the immense amount of labor which this work has involved it will be sufficient to point out that up to the year 1910 over six hundred arsenical products alone had been prepared and tested biologically and therapeutically under Ehrlich's direction. Of these the one carrying the number 606 has been of special interest to clinicians, as its wonderful therapeutic effect upon trypanosome infections and certain spirilloses of animals (notably the spirillosis of relapsing fever and chicken spirillosis) suggested the idea that the product might be similarly effective in the treatment of human syphilis (see Plate IV). After preliminary studies had then shown that a single dose of the substance is capable of causing the complete destruction of all spirochetes in syphilis-infected rabbits, with the complete cure of the testicular chancre and without the occurrence of a relapse, it was clearly indicated that corresponding experiments in the human being were justifiable (see Plate V). After the first trials in this direction had then demonstrated a similarly beneficial effect, Ehrlich placed the remedy in the hands of a large number of special workers in this field in order that a conclusion should be reached as soon as possible regarding its therapeutic value, the indications and contra-indications to its use, the question of dosage, mode of administration, etc. As a consequence reports on these questions could be collected within a year, covering the administration of the remedy in many thousands of cases, so that in a relatively short time the verdict could be reached that preparation 606, or *salvarsan*, as it is now termed, actually constitutes the most potent remedy which we have at our disposal for the treatment of syphilis; and we would emphasize once more that *this discovery was not the result of accident, but the outcome of carefully planned experimentation, carried to its logical issue.*

SALVARSAN AND ITS USE IN THE TREATMENT OF SYPHILIS

Chemically speaking salvarsan (Ehrlich's "606") is the dichlorhydrate of dioxydiaminoarsenobenzol:



It is a fine yellow powder, easily soluble in water, methyl alcohol, and glycerin, less easily soluble in ethyl alcohol and insoluble in ether. Owing to the readiness with which it undergoes oxidation and gives rise to highly poisonous products, it is marketed in little ampoules from which the air has been removed and replaced with an indifferent gas.

Method of Application.—While the substance was originally injected in acid solution, *i. e.*, merely dissolved in water, this method was found inapplicable owing to the intense pain which followed its use, and at present it is employed practically only in alkaline solution, which is administered intravenously, and should be freshly prepared just before the injection. This is done in a sterile bottle of about 500 c.c. capacity, graduated in 50 c.c., and containing some large sterile glass beads. The salt solution (0.65 per cent.) which is used as solvent and diluent should be *freshly prepared from freshly distilled water* and chemically pure sodium chloride. 30 to 40 c.c. of this are placed in the bottle and the dose of salvarsan added, which then dissolves on vigorous shaking.

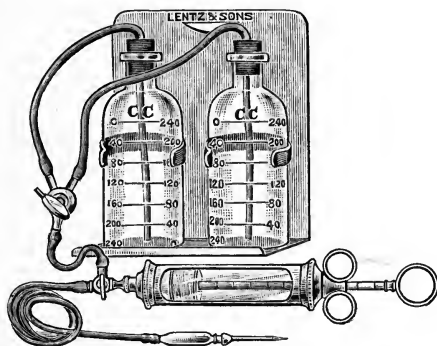
To obtain the alkaline solution 0.19 c.c. of a 15 per cent. solution of caustic *soda* (NaOH) are now added for every 0.1 gram of the remedy, the immediate effect being the formation of a precipitate which dissolves on shaking and then gives rise to a clear golden yellow solution. This is finally diluted with the sterile saline (warmed to body temperature), such that every 50 c.c. shall correspond to 0.1 gram of salvarsan. Taking an adult dose of 0.6 gram as example, the final bulk would thus be 300 c.c.. Should the solution not be absolutely clear a few additional drops of the NaOH solution may be added. Occasionally the fluid does not clear up upon the further addition of alkali, in which event it is probably best to break a new ampoule of the drug.

To give the injection, an ordinary infusion bottle or similar contrivance (properly sterilized, of course) is arranged at the bedside of the patient and charged with a small quantity of warmed salt solution *which should completely fill the rubber tube leading to the needle, as well as the lumen of the latter*. This need not be of large caliber; a No. 18 (B. & S. standard) is quite sufficient in size. The arm having been cleansed, at the bend of the elbow, with soap and water, bichloride, alcohol, and ether, or, has recently been advocated, merely painted with tincture of iodine, about the site of the

injection, the needle is plunged into any one of the large veins which there present themselves, and which have been rendered prominent by constricting the upper arm with a bandage, or a piece of rubber tubing, without, however, obliterating the arterial flow. The tourniquet is then removed, the clamp opened on the rubber tube, and the *saline* allowed to flow. If the result shows that the needle is actually in the vein, the salvarsan solution is added to the small amount of saline remaining in the bottle, and the infusion allowed to proceed. In the end about 50 c.c. of saline are allowed to follow the salvarsan, so that the tissue about the site of the puncture shall be irritated as little as possible in the event of a little leakage while the needle is being withdrawn.

A very convenient apparatus for the administration of salvarsan is that suggested by Cary (Fig. 16).

FIG. 16



Syringe for injection of salvarsan. Glass handle guides operator. Two bottles enable alternate use of drug or water.

Special care should be had that the injection is made slowly, and under no circumstances should it be allowed to consume less time than twelve to fifteen minutes. Want of attention to this point may result in the development of serious symptoms. While it is usual that the patient's face becomes flushed during the injection, the infusion should be stopped at once, if sudden pallor develops, or the pulse becomes weak.

Following the injection the patient should be placed in bed and should remain there for twenty-four hours or longer, according to the symptoms which develop during this time. To give the injection in the physician's office, and then to allow the patient to go home and follow his usual occupation, is a dangerous practice,

unless indeed the dose be small, and the quantity of fluid less than 150 c.c.

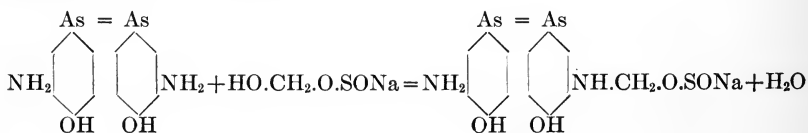
Other methods of administering salvarsan have practically been abandoned, and *we would indeed warn the practitioner against the use of subcutaneous and intramuscular injections*, no matter in what medium the drug may have been dissolved or suspended. *Such injections usually cause a great deal of pain and are not infrequently followed by necrosis.*

Reaction.—The reaction which follows the intravenous administration of salvarsan is essentially of the same character as that following the injection of a corresponding amount of saline, and varies in intensity with the individual case. In many instances the patient is not inconvenienced in the least. At first when it was not known, that it is essential to use *freshly distilled, sterile water* in making up the solution, it was only too common to meet with fairly severe reactions, viz., chills, fever, vomiting, diarrhea, etc., but such symptoms are now seen only exceptionally. Symptoms on the part of the nervous system, notably in connection with some of the cranial nerves, which develop in relatively rare instances, either during the first few days or only after several months, following the use of salvarsan, are to be attributed not to any toxic action on the part of the drug, but to the localization of the spirochetes, and constitute an indication for the further use of the drug rather than the reverse (see Neurorelapses).

That the known contra-indications to the use of the salvarsan should, of course, be considered in connection with every case goes without saying (see below).

Neosalvarsan.—Since salvarsan was first placed upon the market, Ehrlich has attempted to modify the product so that its preparation for injection would be simplified and the use of the caustic alkali, in particular, eliminated, as some of the objectionable features which are at times noted after the injection could be shown to be due to this factor. The result is the so-called neosalvarsan.

This is a condensation product of salvarsan and hydraldite (formaldehyde sulphonylate of sodium), the reaction taking place according to the equation:



Like salvarsan the new product is a yellow powder, which is readily soluble in water, but unlike the original it forms a neutral solution so that no addition of alkali is necessary before use. As its "initial" toxicity at the same time is less (1 gram corresponding to 0.66 of salvarsan), and its spirillicidal action even more intense than that of salvarsan, still better results may be anticipated from its use.

The solution should be prepared *just before injection*, but it is necessary to use a salt solution of lower concentration, *i. e.*, one not stronger than 0.4 per cent., as otherwise it will be turbid, and apparently more toxic. As in the case of salvarsan, moreover, *only freshly distilled or sterile water should be used*. While the temperature of the saline may be as high as 20° C., *the liquid should not be heated after solution has taken place*, for fear of causing the formation of poisonous oxidation products. For the same reason, it is recommended *not to shake the solution unnecessarily*. Older solutions turn a reddish color, and the same is seen in the case of the powder itself, if the ampoule was not absolutely air-tight; such preparations are dangerous and should be discarded.

For injections, 0.6 to 1.5 grams may be dissolved in 200 to 250 c.c. of saline (for dosage see below).

Under no circumstances should the neosalvarsan be used subcutaneously or intramuscularly, as the chances of its oxidation and hence of a material increase in its toxicity would thus be much increased. Nor is it admissible, even if the drug is to be administered intravenously, to prepare the solution in bulk for several patients, as the time interval elapsing between the first and subsequent injections might be sufficient to cause the oxidation of the drug. The general idea underlying the administration of the neosalvarsan is thus to cut down the time exposure of the drug or its solution to the air to a minimum.

Reaction.—The reaction symptoms which follow the use of the neosalvarsan are usually insignificant, while neglect of the instructions just given may be expected to produce the symptom-complex of acute arsenical poisoning. With large doses exanthems have been observed between the eighth and the twelfth day, which may be avoided, however, if smaller amounts are given.

Dosage and Frequency of Injection.—As the injection of any spirillicidal drug that does not effect the complete destruction of all

the parasites at a single dose is apt to lead to the development of serum and drug-fast strains, a large dose, *cæteris paribus*, is preferable to a small dose; if, however, a large dose is for any reason not advisable, it is probably best to inject smaller quantities at brief intervals.

While 0.5 gram is generally recommended as the initial dose of *salvarsan* for men, and a slightly smaller amount (0.3 to 0.4 gram) for women, some investigators have used larger quantities without observing any detrimental effects. In subjects that are not in robust health, or in individuals where one is in doubt whether to use the remedy at all, it is best to give a small initial injection, say of 0.2 gram. and to repeat this dose in a few days if no unusual symptoms develop. In young babies up to the fourth month the dose should not exceed 0.02 to 0.03 gram, while in children of nine or ten years of age 0.1 and 0.2 gram may be given, which may be injected into the gluteal muscles, the amount of liquid being, of course, proportionately smaller. The pain which develops after the injection may be controlled, to a certain extent at least, by hot compresses. But, as I have pointed out above, one must not be surprised if local necrosis develops after the use of the remedy in this manner.

If the *neosalvarsan* is to be employed, 1.5 gram may be given to men and 1.2 gram to women, but it is recommended not to start with these doses, but to give a primary injection of 0.9 gram; to allow a day to intervene and then to inject 1.2, then on alternate days, *i. e.*, with a day of rest intervening, 1.35 gram and finally 1.5 gram. That the dosage can be pushed, however, is shown by the fact that in robust men 6 grams of *neosalvarsan*, corresponding in arsenical content to 4 grams of *salvarsan*, have been given within seven days.

Babies are given 0.05 and children 0.15 gram.

Small initial doses of either preparation are indicated whenever there is reason for assuming the existence of syphilis of the central nervous system and its meninges. As the latter are known to be involved quite early in the course of the infection already Ehrlich suggests that in early secondary cases (especially during the roseolar stage), particularly when nervous symptoms of any kind exist, the *average* initial dose (of *salvarsan*) should not be higher than 0.3 gram, and that in suspicious cases it may be best to begin with 0.1 to 0.15 gram. This dose should preferably not be exceeded even at the

second injection, and if threatening symptoms exist it may even be better to begin with a course of calomel and to follow this up with a very small dose of salvarsan. Our final aim, however, should be the injection of full doses.

Should by any chance symptoms denoting grave danger develop notwithstanding this mode of procedure, lumbar puncture is to be performed without delay, and if no meningeal involvement then manifests itself (as evidenced by the presence of a small amount of fluid only), this is to be followed by trephining. Above all there should be no delay in carrying out such treatment.

A month after the last injection the Wassermann test is made. If this still shows a positive reaction the salvarsan treatment should be repeated, and may advantageously be followed by a brisk course of mercury. If the latter is used, a month should then elapse during which no medication whatever is employed, before the next test is made, and so on, until a negative reaction is reached. From this point off the Wassermann is repeated at more and more distant intervals for from two to three years (see Wassermann Reaction).

Contra-indications to the Use of Salvarsan.—At the very beginning of its use Ehrlich emphasized the importance of excluding all those cases from the salvarsan treatment in which there was reason to assume the existence of advanced disease of the heart or of the central nervous system; particularly cases of angina pectoris, aneurysm (notably of the cerebral vessels), advanced paresis, and cases of atrophy of the optic nerve, while in other syphilitic diseases of the eyes as well as in advanced syphilis of the abdominal viscera the remedy may be advantageously employed. If any doubt exists in an individual case, whether the remedy should be used or not, and the condition of the patient so far as the syphilitic process in itself is concerned makes this desirable, a careful attempt may be made with a very small dose (0.1 gram), which, if no disturbing symptoms develop, may then be followed up with one of equal size or even a little larger (see above, Dosage).

After all we must remember that the number of deaths which can be attributed to the salvarsan itself, or to its effect upon the syphilitic process, and not to harmful technique, is ridiculously small in comparison with the enormous number of cases where no harmful result has followed its use, and where, on the contrary, the greatest amount of good has been accomplished. As Ehrlich has

pointed out, the toxicity of the salvarsan is distinctly less than that of mercury.

Neurorelapses.—Not infrequently certain functional disturbances have been noted to occur in connection with some of the cranial nerves, which appear very soon after the injection. Ehrlich is inclined to look upon these as corresponding to the so-called Herxheimer reaction, which is so frequently observed in the skin soon after the use of salvarsan, and which he refers to the liberation of toxins from the killed spirochetes and to their local irritating effect. He points out that if such a reaction should affect one of the cranial nerves at a point where this passes through a narrow, bony canal, disturbance in function would be a very probable consequence, owing to swelling and resultant compression. Such disturbances, however, do not occur within a few hours of the injection, as in the case of the true Herxheimer reaction, affecting the skin, but only after twenty-four hours, or even after three or four days, as the vascular supply of the nerves is but little developed, and a longer time must elapse before a sufficient number of spirochetes has been killed to produce a local reaction of moment. Owing to the same cause, an opportunity is afforded in these localities for the escape of some of the spirochetes and their subsequent development. Should the spirochetal focus be very small in comparison to the size of the nerve at the point in question, so that no pressure would result in consequence of the first Herxheimer reaction, there will, of course, be no occasion for the development of acute symptoms. But if, then, the surviving spirochetes increase in number a basis would be furnished for what is now commonly termed a *neurorelapse*.

When these relapses, which usually occur two or three months or even four or five months after treatment, were first observed, following the use of salvarsan, they were attributed to the contained arsenic and were supposed to constitute a special danger attending its use. But as Ehrlich has pointed out, the same occurrences have been noted in connection with the use of mercury, and to judge from the collective reports of Benario they are no more frequent after the use of salvarsan than after that of the latter, and here as there the same nerves are especially prone to attack, viz., the auditory, the optic, the facial, and the oculomotor, while the fourth, fifth, sixth, and twelfth are much less frequently affected.

Ehrlich emphasizes in support of his view that neurorelapses only

occur during that period of the disease when there is a maximal distribution of spirochetes, viz., during the early secondary stage, notably in connection with the first exanthem, while during the later stages when actual nerve lesions exist they are not observed. He regards their occurrence as evidence of a nearly complete sterilization of the body, and very aptly compares the neurorelapse to the extensive development of individual bacterial colonies on agar plates, when but few organisms are present, as contrasted with their tiny size when the number is large. He accordingly advises that such cases be reinjected with the salvarsan, and there are already a number of reports to show that such treatment is indeed frequently followed by a most favorable result, while it is well known that a nerve that has actually been damaged by arsenic itself (atoxyl for example) is hopelessly doomed if a second injection is given.

Results.—While there is evidence to show that the *therapia magna sterilisans*, *i. e.*, the complete destruction of all the parasites during a single course of treatment, is no mere dream but an actual possibility, this point is not reached so readily in syphilis of the human being, at least, as in the spirillosis of chickens, in relapsing fever, and in frambesia, where a single injection is usually sufficient. That a cure can be effected, however, in a relatively short time is beyond all question.

Here as elsewhere in the treatment of disease the best results will be obtained if this is instituted early. Gennerich thus reports, that of 58 cases of primary disease that had been treated with calomel, followed by salvarsan, not a single one developed secondaries, nor did the Wassermann reaction become positive again, and that of these, 20 had already been followed for from nine to sixteen months at the time of writing. Tänzer, who used the salvarsan by itself, similarly reports that of 21 cases which could be followed for from three to thirteen months, none had a relapse, while the Wassermann reaction remained negative. Arning states the same of 67 cases which had been treated with salvarsan and mercury, etc. The question, of course, might rightfully be asked, whether these people could actually be regarded as cured, and whether the disease had not merely become latent. Opposed to such a conclusion is the fact that some cases of syphilis which had been treated with salvarsan, and in whom no further symptoms developed, later came back with a new infection, *i. e.*, with a new chancre, which would prove that the patient must

actually have been cured, since reinfection in the active syphilitic is not possible. To this it might be objected that the new chancre was in reality not a new infection but a relapse, analogous to the neurorelapses referred to above. But as Ehrlich remarks, the neurorelapses occur after a period of from two to five months, so that if no symptoms develop within that period of time, one would hardly be justified in looking upon the cases referred to above as being latent.

The assumption that a cure had actually been established is, however, further supported by the fact that in those cases which could be examined in this direction a so-called *provocative Wassermann reaction* could not be elicited. This reaction is based upon the idea that in individuals in whom the spirochetes have been exterminated to such a degree that a positive Wassermann can no longer be demonstrated but in whom a small number of organisms still remain, this may yet be done, temporarily at least, if a further injection of the salvarsan is given, or if a few large doses of mercury are administered. As a matter of fact, it can be shown that in truly latent cases a positive Wassermann may indeed be obtained in this manner in the course of two weeks from the time of the test treatment, and Ehrlich very properly advises that such an examination should be made before a patient is finally discharged from treatment.

Further evidence of the remarkable efficacy of the salvarsan treatment is the rapidity with which the spirochetes disappear from primary sores and from secondary ulcerations. This usually takes place within twenty-four hours, but sometimes even more rapidly. Schreiber thus mentions a case that had been treated with neo-salvarsan, in which the organisms could no longer be demonstrated four hours after the injection. He nevertheless advises the excision of the primary sore whenever this is possible.

While the best results may thus be expected during the primary stage of the disease, especially if several doses of salvarsan, possibly followed by mercury, have been administered, no effect whatever is to be hoped for in cases that are no longer suffering from their syphilis proper, but from the consequent lesions. Symptomatic improvement, to be sure, may at times be seen even then, and is no doubt due to the destruction of the few foci of spirochetes that may still be remaining, and the elimination of such sources of toxin production. But upon the symptoms that are the outcome of the actual destruc-

tion of important cell complexes, such as the blindness and ataxia of tabes, the remedy will naturally be without effect. It is to be noted, however, that in very early cases one may occasionally see remarkable improvement even in some of those very symptoms which we are accustomed to refer to the actual destruction of nerve cells, so that the inference suggests itself that *some* of the symptoms, of tabes, for example, may be due both to toxic influences and to an actual destruction of nerve cells. For this reason the remedy may be given a trial in tabes and paresis at the first sheet lightning, as Ehrlich puts it, while later on it is, of course, useless, and in advanced paresis especially, its employment is even attended by a certain amount of danger.

As the destruction of spirochetes leads to the production of antibodies of a protective character, as is evidenced by the beneficial effect which the milk of salvarsan-treated syphilitic mothers has upon the syphilitic lesions of the child, it has been suggested to extract blood from treated syphilitic patients and to inject the serum into the subarachnoid space of such cases of cerebrospinal syphilis (paresis) in which salvarsan is of no benefit, when given by itself. But as yet no data are available to warrant any conclusions regarding such a mode of procedure. It would seem logical, but it may be questioned whether the injected antibodies would reach the spirochetes in sufficient quantity to do much good.

However this may be, if we eliminate from our analysis all those cases in which destructive lesions have occurred, and sum up the findings in the remainder, there is overwhelming evidence to show that in salvarsan, either by itself or in combination with mercury, we have a treatment by which we cannot only produce a favorable influence upon the clinical symptoms, but actually effect a cure, in the vast majority of cases. It seems very doubtful in fact whether any cases exist, in which the infection cannot be completely eradicated either by the salvarsan alone, or in combination or alternation with mercury, if the results of the treatment are controlled at frequent intervals by the Wassermann reaction, and if the treatment itself is carried out by experts. A suitable combination of the syphilologist's clinical knowledge and the peculiar training of the immunologist will unquestionably yield the best results; either alone is not in a position to give the patient the very best that can be given.

To enter into a detailed account of case records would lead us

too far afield, however, and I would refer those who are interested to the special literature upon the subject; suffice it to say at this place that barring those cases in which the treatment is clearly contra-indicated, it should be followed whenever there is reason to believe that living spirochetes are present in a patient's body, as evidenced either by the character of the clinical symptoms or the presence of a positive Wassermann reaction.

SALVARSAN AND ITS USE IN NON-SYPHILITIC MALADIES

While salvarsan has gained its greatest fame in the treatment of syphilis, there is evidence to show that the remedy is even more effective in combating other infections that are due to protozoan parasites. It has thus been found of signal value in the treatment of those cases of tertian *malaria* which are refractory to quinine. In this connection the interesting observation has been made that in some cases of this order the administration of salvarsan in very small doses may cause the refractory behavior to quinine to disappear.

Brilliant results have been reported by many observers in the treatment of *relapsing fever*, where a single injection suffices to cause the parasites to disappear and to effect a lasting cure. Equally favorable results have been obtained in *frambesia*, which plays a more important role among the plantation workers of Surinam than even syphilis. Koch and Flu report that of 900 cases which had thus been treated only three developed a relapse. As a rule a single injection is sufficient, or at most two injections. Quite important, further, is the observation of Joannides that *bilharziasis* can be cured with a single injection. The same is reported concerning the effect of the treatment on *aleppo boil*, while it seems to be of no avail in *kala-azar*. Whether or not the remedy is of use in the treatment of *typhus fever* is not yet certain; some writers report favorable results, while others are less enthusiastic. In *amebiasis* and *Vincent's angina*, however, it seems to have a definitely favorable effect. Wonderful results have been reported in *yaws*. In the treatment of *sleeping sickness* the results have been inconstant. As the tendency to the development of arsenic-fast strains is much greater in the case of the trypanosomes than in the spirochetes,

every attempt should here be made to destroy the parasites with a single dose, while the *therapia fractionata*, which is to a certain extent permissible in syphilis, is less apt to be successful.

CHEMOTHERAPY IN BACTERIAL INFECTIONS

While the application of the new science of chemotherapy to the study of protozoan infections has thus led to most brilliant results within the few years of its existence, the thought naturally suggests itself whether some of the bacterial infections also may not be amenable to medicinal treatment upon this basis. *A priori*, of course, this possibility exists, but it is noteworthy that the only diseases in which a specific cure could be effected in the olden days of medicine were of protozoan origin, *i. e.*, malaria and syphilis, and it is to be feared that the problems are much more complicated in the bacterial infections. There is some evidence to show, however, that here also a new era of treatment may be expected to dawn in the near future and that modern pharmacology when approached from the standpoint of general biology may succeed in accomplishing what the pharmacology of the olden days failed to do. A more intimate knowledge of cell metabolism and above all of cell nutrition will unquestionably carry with it the solution of the problem of bacterial infections. At this place I would only briefly refer to the recent advances in the chemotherapy of pneumococcus infections. Lamar has thus shown in the animal experiment that while a corresponding immune serum is incapable of preventing the development of pneumococcus meningitis when introduced subdurally by itself, a mixture of sodium oleate, immune serum and boric acid regularly exerted a more powerful influence than the immune serum alone, and not only prevented the occurrence of infection, but also, when administered separately, arrested the progress of an actually established infection, and led, often, to the enduring and perfect recovery of the animal.

On the basis that a certain parallelism in their biological behavior exists between trypanosomes and pneumococci, and that certain quinine derivatives were found to have a trypanocidal effect, Morgenroth and his collaborators undertook corresponding studies in animal infections with the pneumococcus. As a result of their investiga-

tions they found that whereas quinine, hydroquinine, and hydrochlorisoquinine had no effect upon the course of artificial pneumococcus infections, ethyl-hydrocuprein was capable of arresting the infection in 50 per cent. of their animals, when given six hours after the inoculation, *i. e.*, at a time when from ten to a thousand multiples of the fatal dose of organisms were already in circulation. Although corresponding experiments in the human being have not as yet yielded encouraging results, the data obtained in the animal experiment are highly significant, as they have clearly demonstrated that the substance in question has a selective affinity for the organisms concerned. For this reason it would seem quite within the domain of possibility that a substance may be produced which may be more effective and freer from undesirable side effects, or, to use the parlance of Ehrlich, one in which the bacteriotropism would be greatly predominating over its organotropism.

CHEMOTHERAPY IN MALIGNANT DISEASE

While the pathogenesis of malignant disease is still *sub judice*, and while no satisfactory evidence has as yet been furnished to support the belief that it is parasitic in origin, it would seem as though the principles which are involved in its non-surgical treatment, are after all the same as those with which we have become familiar in the course of our study of the infectious diseases proper. Here as there cells are multiplying within the body, which in malignant disease are in a manner just as foreign to the adjacent normal cells as a bacterium or a protozoan parasite would be, and here as there the life of the foreign cell in its abnormal environment leads to disturbances of the normal functions not only of the adjacent tissues, but of the body at large, which may be so serious in character that the death of the macroörganism may follow, and in malignant disease indeed invariably follows. Here as there then the main plan of our treatment must be to so influence the proliferating foreign cell as to lead to its direct destruction, or at least to prevent its development, without causing undue harm to the normal cells of the body. Evidently this is in part at least a problem of modern pharmacology and one which is intimately connected with the study of immunity; for we must remember that cell destruction within

the body, of whatever kind, will invariably lead to a response on the part of the macroörganism which is in the end of a protective character. It may accordingly not be out of place in a book of this character to briefly review some of the more promising lines of investigation in the domain of chemotherapy which are at the present time occupying the attention of students of the cancer problem. That the problem should be intrinsically more difficult in malignant disease goes of course without saying, for it is here not only a question of finding a substance which would have a low grade of affinity for the body cells coupled with a high grade of affinity for a cell which is a perfect stranger to the macroörganism, but we must actually find a product which shall have a high affinity for a type of cell which after all is a product of the same organism against whose normal cells, so to speak, its affinity must be low. The possibility of actually finding a substance of this character will naturally depend upon the question whether the proliferating cell, which for want of a better term we may speak of as the cancer cell, is structurally and functionally identical with the normal cell from which it has originated, or whether any points of difference exist between the two types. While this question still awaits its final answer, we may say that there is some evidence to show that points of difference do exist between the normal and the abnormal cell, and that the problem is hence *a priori* not a hopeless one.

The most interesting studies which have a bearing upon the possibility of effecting a cure of cancer along the lines of modern chemotherapy have been published by A. v. Wassermann and his collaborators.

The investigations of these observers were based upon the discovery by Gosio that sodium selenate and sodium tellurate are more rapidly reduced by cancer cells than by normal cells, and that this reduction takes place within the bodies of the cells. Experiments on tumor mice then showed that the injection of these substances into the growths may actually lead to their complete destruction. The problem now was to devise some method by which the selenium or the tellurium could be carried to the tumor through the circulation in order to prove that the substance in question actually possesses a selective affinity for the tumor cell, and remembering also that the complete eradication of the malignant growth, excepting in the very earliest stage of the disease, can scarcely be expected by means

of local or even regional treatment. Considering the meager blood supply of epithelial tumors more especially the idea naturally suggested itself to combine the selenium or tellurium with some substance endowed with great powers of diffusion, and to utilize this as a carrier of the cell-destroying metal. As eosin possesses such properties this was selected and a number of eosin-selenium compounds tested out in this direction. While the results obtained were not uniform, a definite cure was nevertheless achieved in several animals, and with the demonstration of this fact the cancer problem seems to have been solved in principle, and it has been proved that not only a growing tumor can be caused to retrogress entirely in consequence of the introduction into the body of a definite chemical compound by the intravenous route, but also that this is possible without causing undue harm to the body at large.

Of the *modus operandi* of the selenium we are as yet in ignorance. Neuberg and Caspari have expressed the opinion that the injection of certain compounds of the heavy metals, notably when administered in colloidal form, favors the self-digestion (autolysis) of the tumor cell, and they succeeded in demonstrating that there are actually substances which possess a selective affinity for cancer cells, and that in cancer mice it is possible to bring about the dissolution of the tumor with such "tumor affinic" substances. They tested out a large number of the heavy metals and could verify Wassermann's observation that selenium also possesses such properties. They found, however, that copper and tin are even more active in this respect. These experiments have given rise to most active work along these lines, and sufficient evidence has already accumulated to warrant the hope that ere long a method may be devised which may be applicable in the human being also. Some work in this direction is indeed already in progress, but it would be premature to draw any inferences from the little that has as yet been done.

In conclusion we would yet briefly refer to the work of R. Werner and his collaborators. Starting from a certain parallelism which exists between the effect of radium and x -ray applications and the action of certain cholin salts upon the leukocytes and the reproductive glands, and the fact, moreover, that there is some evidence to show that the lecithin of the cells is decomposed by the x -ray and radium emanations, with the liberation of cholin, these investigators expressed the opinion that the beneficial effects of radium and x -ray

treatment upon malignant growths also might very well be due to the liberation and action of these very substances. They accordingly studied the effect of various cholin preparations upon tumor growth, and found as a matter of fact that the changes resulting in the tumors were perfectly analogous to those produced by ray treatment. Since cholin could be shown to possess a high power of diffusion the thought then suggested itself, in view of the observations above related concerning the selective action of colloidal metals upon tumor cells, to use the cholin as carrier of these metals. Studies in this direction showed that beneficial effects may indeed be thus achieved in the animal experiment with colloidal selenium, copper, cobalt, iron, zinc, and arsenic, when dissolved in cholin solutions, and that this could be accentuated by combining a metal of high electrical tension with one of low tension, or even with one which in itself is inactive, such as selenium-vanadium, selenium-cobalt, copper-cobalt, etc. There is thus still another avenue open for attack, and one which likewise seems to promise profitable results.

CHAPTER XV

THE APPLICATION OF IMMUNOLOGICAL PRINCIPLES TO DIAGNOSIS

WHILE in the foregoing chapters we have been interested largely in the reaction of the animal body to the introduction of alien cells and cell products, from the standpoint of therapy, it is important to note that some of the principles involved in these reactions have also found application in the diagnosis of many of the infectious diseases. The recognition of the formation of agglutinins has thus led to the discovery of the most important method in the diagnosis of typhoid fever, paratyphoid fever, and Malta fever; the principle underlying the formation of bacteriolysins is utilized in the diagnosis of cholera; the formation of special antibodies, which in the presence of corresponding antigen absorb complement, and which may thus be recognized indirectly by the demonstration of such complement fixation, serve as a basis of the Wassermann diagnosis of syphilis; the recognition of the general allergic state in the sense of v. Pirquet has led to some of the most important methods in the diagnosis of tuberculosis and syphilis; the precipitins play an important role in the recognition of specific albumins, and serve as a basis of the modern tests for blood in legal medicine; the formation of antiferments has been utilized in the diagnosis of cancer, etc.

While a detailed account of all the *immunological methods of diagnosis* would lead us too far, and would indeed furnish sufficient material for a special volume, it may not be out of place to consider a few of the more important methods of this order in some detail.

THE AGGLUTINATION REACTION

In 1896 Gruber and Durham pointed out that the addition of cholera and colon immune serum to bouillon cultures of the corresponding organisms produced a remarkable effect, for on standing for

a number of hours the turbidity of the cultures disappeared; while all the bacteria had settled to the bottom. This peculiar behavior, as we now know, is owing to the presence, in the sera in question, of certain antibodies known as agglutinins which are formed as a result of infection (sc., immunization), and are characterized by the fact that in suitable dilution they will cause the arrest of motility and agglutination of the corresponding organisms (see also section on Antibodies). Normal serum, it is true, will also do this to a certain extent, but only when used in a fair degree of concentration, and then only imperfectly, while with immune sera the complete reaction may be obtained even though the serum be diluted many times. In this sense the reaction is specific, and may be employed both for the identification of a given organism as also for the recognition of the nature of an immune serum. In the first instance an emulsion of the unknown bacterium is brought together with diluted test sera, corresponding to those organisms which would enter into consideration from a diagnostic standpoint. If, then, the bacterium in question is agglutinated by an antityphoid serum, for example, but not by an anticolon or an antidyentery serum, the inference would be (within certain experimental limitations) that we are dealing with the typhoid bacillus. On the other hand the unknown serum, in a certain degree of dilution, is tested against a series of organisms, when a positive result with one of these would indicate the nature of the infection. From both standpoints the agglutination reaction has thus a wide sphere of application.

Very soon after the discovery of Gruber and Durham, Widal found that the formation of agglutinins in typhoid fever begins quite early in the course of the disease, *i. e.*, at a time when from the usual symptoms the diagnosis cannot as yet be made with certainty, and he thus established a method of diagnosis which in some one of its numerous technical modifications is now used the world over, and is generally known as the *Widal reaction*. Further studies, then, showed that the formation of agglutinins in other infections likewise begins while the disease is in actual progress, and that the same principle may be successfully utilized for diagnostic purposes in a number of other maladies besides typhoid fever. This is notably the case in paratyphoid infections, in Malta fever, and in meningococcus infections. In other maladies agglutinin formation also takes place, but either does not begin early enough to be of service in

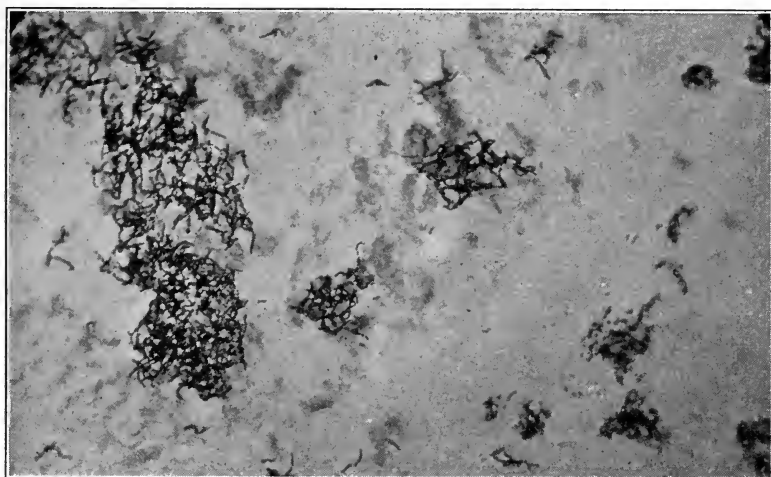
diagnosis (as in plague, for example), or there are certain technical difficulties which make it inapplicable (tuberculosis, cholera), while in still others a diagnosis can be conveniently reached in an even more direct manner (as by the isolation and cultivation of the offending microörganism), etc.

To give a general idea of the *technical method of procedure* it will be best to describe the reaction as it is applied to the diagnosis of typhoid fever, *i. e.*, the Widal reaction proper.

The Widal Reaction.—TECHNIQUE.—*Microscopic Method.*—A small amount of blood (5 to 10 drops) is collected in a little glass tube or in one of the capsules pictured in Plate III. The serum is separated by centrifugation and a drop diluted in the white mixing pipette accompanying the hemocytometric counting chamber in the proportion of 1 to 20. From this, subdilutions of 1 to 40, 1 to 80, 1 to 160 are prepared with the aid of the same pipette, normal salt solution being used as diluent in all cases. Four slides are then ringed with vaseline, and into each little chamber a drop of the diluted serum is placed together with a drop of a typhoid culture in bouillon, not more than twenty-four hours old. The resultant dilutions will then be 1 to 40, 1 to 80, 1 to 160, 1 to 320. A cover-glass is adjusted so as to be in contact all around with the vaseline, as also with the drop in the central chamber. The specimens are immediately examined with the middle power of the usual microscopic outfit ($\frac{1}{6}$ B. and L.; No. 6 or 7 Leitz), and discarded if any large clumps of bacteria are seen. Should this be the case, it is well to make new mounts with a culture that has been centrifugalized for a minute or two, the supernatant fluid only being used, in which no clumps will be found. If the mount is satisfactory, it is set aside and reëxamined at the expiration of half an hour. If the reaction is positive, all the bacilli will be found motionless at the expiration of this time, and gathered in clumps of variable size (Fig. 15). This will be the case at least in the lowest dilutions, while in the higher ones it may be necessary to wait until another half-hour has expired. The higher the dilution in which complete clumping may be obtained the greater is the diagnostic significance of the reaction. Ordinarily, complete clumping at the end of half an hour in a dilution of 1 to 40 is sufficient; if, however, the question of paratyphoid enters into consideration, the result in the higher dilutions should be considered. As the typhoid and paratyphoid bacilli carry certain receptors in common, the

corresponding antisera also will contain certain agglutinins in common which can unite with both types of organisms, and thus give rise to agglutination in the lower dilutions. As the more specific receptors, however, predominate over those that are common to both types, the corresponding agglutinins will also be more abundant in the antisera, so that the type of infection can be established from the higher dilutions in which a serum will cause agglutination of a given organism.

FIG. 17



Positive agglutination reaction.

A material advance in the practical applicability of the Widal reaction was achieved when it was discovered that it is not necessary to work with living cultures of the typhoid bacillus, but that dead organisms will answer just as well, providing that the strain was readily agglutinable before being killed. To this end it is convenient to prepare a bouillon culture in an Erlenmeyer flask, to incubate for twenty-four hours at 37° to 40° C., and then to add 40 per cent. formalin solution (*i. e.*, the concentrated solution of the Pharmacopœia) to the extent of 1 per cent. After standing for two to five days in the incubator the emulsion is centrifugalized, the bacilli are washed with two changes of sterile normal salt solution and diluted to the original volume, when the fluid emulsion may be

preserved in sterile glass beads in the ice-box. In this manner the material will keep for months, and can be used either for the microscopic test, in which case the time of examination should be extended to two hours, or it may be employed in the macroscopic test described below.

Macroscopic Method.—This method is just as exact as the microscopic method, and is in a manner less apt to lead to confusion; somewhat larger amounts of blood, however, are required (1 c.c.). The serum is diluted in the same proportions as described above. Equal quantities (0.25 c.c.) are then placed in small tubes, such as the collecting tubes figured in Plate III, and treated with equal volumes of the bacterial emulsion. These tubes together with a control of equal volumes of saline and bacterial emulsion are placed in the incubator, or some other warm place, and are examined after twelve to twenty-four hours. If the reaction is positive the bacteria in the serum tubes will have settled to the bottom, leaving the supernatant fluid almost clear, thus contrasting sharply with the control which is still as turbid as it was in the beginning.

RESULTS.—While a positive Widal reaction may be obtained as early as the first day of the disease, meaning thereby the first day that the patient spends in bed, or the fifth of general malaise, such an occurrence must be viewed as a great rarity. In the vast majority of cases a positive result is obtained only after the fifth or sixth day in bed. As the likelihood of positive bacteriological findings is greatest during the first week of the disease, an examination in this direction may at this time well take precedence over the agglutination test. During the second week, when the value of the two methods is on a par, convenience may decide which one is to be employed. After this, however, the agglutination test should be given the preference. Experience has shown that a positive reaction may be obtained in practically all cases of true typhoid fever, but it is clear from what has been said that much depends upon the period of the disease at which the examination is made. The production of agglutinins evidently does not begin at the same time in all cases, and does not become fully established until after the disease has progressed for a certain length of time. It may happen, indeed, that a positive reaction is not obtained until convalescence, or even until a subsequent relapse occurs. For this reason it is advisable to repeat the examination at frequent intervals, if on first trial a negative result is obtained.

Intermittence of the reaction, moreover, is quite common and emphasizes the necessity of frequent examinations still farther.

While in some instances the reaction disappears very soon after the temperature has returned to normal, and even earlier, it generally continues well into convalescence, and may, in some instances, be obtained after months and even years following the attack. In a series of 71 post-typhoid cases, Krause found the reaction in 36, viz., in 16 of 26 cases examined within a year, in 12 of 21 examined between the second and the fifth year, in 7 of 19 between the fifth and the tenth, and in 1 case out of 5 between the tenth and twentieth (twelfth) year. In three instances no reaction could be obtained within a month of the disease. To what extent the continued presence of typhoid agglutinins may be referable to the persistence of the corresponding bacilli in the body has not been ascertained. It is known that they may persist in the gall-bladder and in the urinary bladder for a long time, and in several instances they have been found where no history of an antecedent typhoid fever could be obtained. In a case of cholelithiasis, reported by Cushing, typhoid bacilli were found in the gall-bladder, and distinct clumping obtained with a dilution of 1 to 30, although the individual gave no history of typhoid whatsoever. Cases, further, are occasionally seen which clinically resemble typhoid fever very closely, but which do not give the Widal reaction at any time, with the usual dilution of 1 to 50. Some of these cases are referable to infection with organisms which are closely related to the typhoid bacillus and which also give rise to the formation of agglutinins. These, however, do not react with the typhoid bacillus excepting in low dilution.

Infection with related organisms may also be responsible for certain cases of febrile jaundice (Weil's disease), in which agglutination of the typhoid bacillus has been observed. In others the reaction may be due to a *localized* infection with typhoid bacilli. The biliary constituents in any event are not responsible for the reaction. This is clear from the observation of Kammerer, who obtained agglutination in only 3 cases of jaundice out of 50 selected at random.

In the diagnosis of paratyphoid, Malta fever, and meningococcus infections a corresponding technique is employed, for a consideration of which the reader is referred to special works dealing with diagnostic methods from the laboratory standpoint.

BACTERIOLYTIC REACTIONS

It will be recalled that Pfeiffer pointed out that cholera vibrios when introduced into the peritoneal cavity of cholera-immune guinea-pigs are there rapidly destroyed through the agency of the normal complement of the animal and the bacteriolytic amboceptor which has been produced in consequence of immunization. The principle underlying this reaction has been recommended for the diagnosis of both cholera and typhoid fever, but is at present only utilized in connection with the former malady, whereas, owing to its greater simplicity, the agglutination test is almost exclusively employed in typhoid fever. This latter test, as we have already seen, is for technical reasons inapplicable in the diagnosis of cholera.

As in the case of the agglutination test, Pfeiffer's reaction also can be utilized either for the purpose of identifying the organism in question, or in searching for the corresponding amboceptor in the serum of a patient.

In the first instance the organism under consideration is suspended in cholera immune serum, and the mixture injected into the peritoneal cavity of a guinea-pig, when the prompt occurrence of bacteriolysis will indicate that the organism was actually the cholera vibrio. In the second case the serum to be tested is inoculated with cholera vibrios and likewise injected into a guinea-pig, when the occurrence of bacteriolysis will prove that the serum contained anticholera amboceptors and was hence derived from an individual who must recently have passed through an attack of the illness in question.

It goes without saying, of course, that serum and organisms must in both instances be combined in certain definite proportions, to which end the following procedure may be employed, as recommended by the Prussian Institute for Infectious Diseases.

1. Pfeiffer's Test as Applied to the Identification of Cholera Vibrios.—For this purpose an anticholera rabbit serum should be available which should be of such strength at least that 0.0002 gram will cause the complete destruction of an oese (=2 milligrams) of an eighteen-hour-old agar culture of the cholera vibrio within one hour after injection into the peritoneal cavity of a guinea-pig.

One pig (A) is then injected with five times the titer dose of the immune serum, *i. e.*, 1 milligram together with one oese (=2 milli-

grams) of an eighteen-hour-old culture of the suspected organism, suspended in 1 c.c. of broth. A second animal (*B*) is given ten titer doses (=2 milligrams) with the same quantity of organisms. A third (*C*) receives 50 multiples of the titer dose, *i. e.*, 10 milligrams, of *normal* serum, however, but taken from an animal of the same species as that furnishing the immune serum, together with the same quantity of organisms as *A* and *B*, while a fourth guinea-pig (*D*) is injected with the same dose of bacteria, but without any serum. The animals should all be of about the same weight (250 grams), and are all injected intraperitoneally. To this end it is recommended to make a small incision through the skin and to inject through a cannula with a blunt point. By the aid of glass capillaries a droplet of the peritoneal fluid is then procured through the same incision, immediately after the injection, a second one twenty minutes later, and a third one at the expiration of one hour. The specimens are examined as hanging drops with an oil-immersion lens. If the organism under consideration is the cholera vibrio, typical granule formation and lysis will be observed in specimens *A* and *B* after twenty minutes already, and at the latest at the expiration of one hour; while in *C* and *D* there will be large numbers of actively motile organisms or such at least in which the form has been well preserved, the *C* animal being the control to *A* and *B*. The object of injecting *D* is merely to prove that the organism in question is virulent and this animal as well as *C*, of course, should die, while *A* and *B* remain alive. If the result then turns out as just indicated, the inference is justifiable that the organism under examination was really the cholera vibrio.

2. Pfeiffer's Test as Applied to the Recognition of Recent Cholera Infections.—In this case the individual's serum is diluted with broth in the proportion of 1 to 20, 1 to 100, and 1 to 500, when guinea-pigs are each inoculated as described above with 1 c.c. of various dilutions, together with one oese (=2 milligrams) of an eighteen-hour-old agar culture of a virulent cholera strain. If extensive bacteriolysis can then be demonstrated at the expiration of twenty minutes, or at most an hour, the inference is justifiable that the person has recently passed through an attack of cholera.

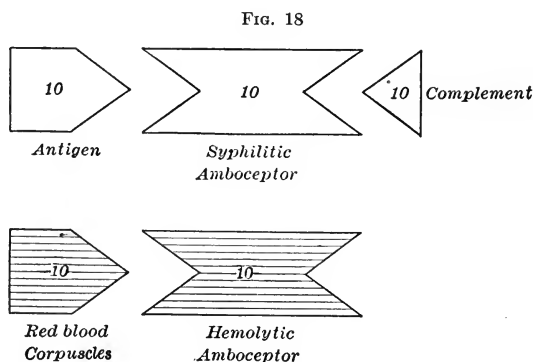
PREPARATION OF THE CHOLERA IMMUNE SERUM.—The cholera immune serum which is required in test 1 (above) is prepared as follows: A number of rabbits are each injected intraperitoneally

with a single cholera agar culture that has been killed by exposure for one hour to a temperature of 56° C. Two weeks later the animals are bled to death, the sera mixed, evaporated in a vacuum, and the dry residue is put up in portions of 0.1 or 0.2 gram, in glass beads, which are then sealed. The titer of this preparation must be ascertained before use; it usually corresponds to about 0.0005 milligram as calculated for the liquid serum.

In lieu of the bacteriolytic test *in vivo*, as outlined above, Stern and Korte have proposed a corresponding method of examination *in vitro*, for a consideration of which the reader is referred to special works.

DIAGNOSTIC REACTIONS DEPENDING UPON COMPLEMENT FIXATION

When reaction products of amboceptor character are brought together with their corresponding antigens in the presence of complement, an interaction takes place between the two first mentioned bodies, in consequence of which complement is bound. This can be demonstrated by the subsequent addition of washed red corpuscles,



and a corresponding hemolytic amboceptor, when hemolysis will either not occur at all, or does so only to a limited extent, according to the degree to which the available quantity of complement has been bound. The exact outcome of the reaction will, of course, depend upon quantitative conditions. If we suppose that the interaction

between the various components takes place according to units, it is clear that if ten units of antigen, for example, were to combine with ten units of the corresponding antibody, and if ten units of complement were absorbed, then upon the subsequent addition of ten units of corpuscles and ten units of hemolytic amboceptor no hemolysis whatever could take place.

If no antibody corresponding to the antigen were present, the ten units of complement would remain free, and could then combine with the ten units of the hemolytic amboceptor, in which case complete hemolysis of the ten units of red cells would take place. Between these two extremes, various grades of hemolysis are, of course, possible, according to the quantity of antibody that is present.

This reaction, like the agglutination reaction and the Pfeiffer reaction, can be used both for the purpose of identifying a given organism as also for demonstrating the presence or absence of certain amboceptors in the blood serum. The recognition of this fact led to the discovery that in syphilis, antibodies appear in the serum which are different from the common bacteriolytic amboceptors, insofar as they will combine with substances that are normal constituents of the body, *i. e.*, certain lipoids. Between the latter and the corresponding syphilitic antibody, however, an analogous reaction takes place, as between bacteria and their amboceptors, in consequence of which complement is absorbed, so that the same principle can be utilized in the diagnosis of syphilitic infections as well. Applied to this end, the reaction is spoken of as the *Wassermann reaction*, as Wassermann was the first to purposely employ the principle as originally understood, to the diagnosis of the infection in question. The discovery of this reaction must rank as one of the most important in the history of medicine, and in its absence the triumphs of Ehrlich's salvarsan could never have been achieved. Its employment, as a matter of fact, forms the basis of the modern treatment of syphilis, and serves as the most delicate indicator of the resultant changes which lead to the recovery of the patient, besides being the most delicate method that we possess for the diagnosis of latent syphilitic lesions.

The Wassermann Reaction.—When Wassermann first applied the principle of complement fixation to the study of syphilitic patients his idea was that antibodies of amboceptor character might be present in the blood serum of such individuals, in which case it should be possible to demonstrate these by bringing them together with

spirochetal antigen on the one hand and complement on the other. As shown in Fig. 18, the complement would then be bound to a greater or less extent as the result of the interaction between the other two factors. Since the cultivation of the syphilitic spirochete had at that time not been accomplished, however, Wassermann was obliged to make use of extracts of organs which were rich in the organisms in question. To this end he employed saline extracts of livers from syphilitic fetuses. With such material as antigen he then actually obtained complement fixation of marked degree, and he very naturally concluded that the reaction which took place was one corresponding to that occurring between a bacteriolytic amboceptor and its corresponding antigen.

Later studies, however, showed that this could not be the case, since identical results were obtained with alcoholic extracts derived not only from syphilitic organs, but from perfectly normal tissues as well. At the present time we know that the reacting substance of the "antigen" is in no sense a specific constituent of the spirochete, but apparently a lipid of the order of lecithin. The syphilitic antibody accordingly cannot be an amboceptor in the sense of Ehrlich, but is evidently a substance which possesses a marked affinity for certain lipoidal bodies, with which it is capable of interacting, with the consequent absorption of complement. Of the nature of this interaction we know nothing. Pending investigations in this direction we may nevertheless represent the process diagrammatically, as I have done above, bearing in mind that in the Wassermann reaction the factors designated as antigen and antibody are so termed only for sake of convenience. For the antibody in question I would suggest the term, *lipoidophilic antibody*, as denoting its essential characteristic and its nature as a reaction product to infection.

PREPARATION OF THE REAGENTS.—1. *Preparation of the Antigen*.—While Wassermann originally advocated the use of saline extracts of syphilitic livers, and other investigators then showed that alcoholic extracts of normal organs (heart, liver, kidney) answer the purpose as well, Noguchi pointed out that the "antigenic" properties of such extracts essentially belong to the acetone-insoluble fraction, and that undesirable "side" reactions can be avoided by utilizing this fraction only. For this reason I have abandoned the use of simple alcoholic extracts altogether, and employ the acetone-insoluble fraction exclusively. This is prepared as follows: Fifty or a hundred

grams of beef heart, liver, or kidney are passed through a meat-hashing machine and extracted with ten times the amount of absolute alcohol by standing for several days at incubator temperature. The resultant mixture is filtered through ordinary filter paper, the filtrate evaporated to dryness with the aid of an electric fan, the residue taken up with as little ether as possible, and the ethereal solution treated with five times its volume of acetone. A precipitate forms, which is allowed to settle to the bottom, when the supernatant fluid is poured off. From the remaining brown, sticky material a saturated solution is prepared in absolute methyl alcohol, which is conveniently put up in glass beads or ampoules, in quantities of about 1 c.c. each.

Prepared in this manner the antigen keeps for many months without losing in strength, but should be tested from time to time nevertheless. To this end emulsions of varying strength are prepared with 0.9 per cent. saline, treated with constant amounts of complement, incubated for thirty minutes in a water-bath at 37° to 40° C., and then combined with the hemolytic system that has been chosen to ascertain whether complement fixation has taken place or not. The general plan to be followed is shown in the accompanying table:

Antigen dilutions in saline. c.c.	Saline 0.9 % solution. c.c.	Guinea-pig complement (1 in 10). c.c.	Washed sheep corpuscles (5% emulsion). c.c.	Hemolytic amoebocyte (2½ times the titer strength). c.c.	Result.
0.5 (4.0 in 10)	0.5	0.5	0.5	0.5	No hemolysis.
0.5 (3.0 in 10)	0.5	0.5	0.5	0.5	Partial hemolysis.
0.5 (2.5 in 10)	0.5	0.5	0.5	0.5	Partial hemolysis.
0.5 (2.5 in 10)	0.5	0.5	0.5	0.5	Partial hemolysis.
0.5 (1.5 in 10)	0.5	0.5	0.5	0.5	Complete hemolysis.
0.5 (1.0 in 10)	0.5	0.5	0.5	0.5	Complete hemolysis.

As the antigen in itself is capable of absorbing a certain amount of complement it will be found that with the stronger emulsions either no hemolysis at all occurs, or partial hemolysis only takes place. For the actual experiment two-thirds of that strength is chosen which first gives complete hemolysis. In the above example it will be noted that this was first obtained with the 1.5 in 10 dilution; in this case then we would use the antigen in a dilution of 1 in 10, and this

would represent its titer. After this has been ascertained the antigen (in the dilution just determined) is next tested against a known syphilitic serum and a known normal serum, both having been inactivated by heating for thirty minutes in the water-bath at 56° C., and extracted with sheep corpuscles, as described below (sub. 5), 0.5 c.c. of the diluted serum being substituted for the 0.5 c.c. measure of saline, corresponding to the second column in the table above. With the known syphilitic serum no hemolysis should then result, while with the normal serum hemolysis must be complete at the expiration of thirty minutes in the water-bath. Occasionally it happens that partial fixation of complement occurs with *normal* serum even. Such antigens are evidently not fit for use, for although every serum possesses anticomplementary properties to a certain extent, it would be dangerous to let the boundaries of the normal and the abnormal overlap. In testing out the antigen it is further well to set the tube, in which complete fixation was noted at the expiration of thirty minutes, aside in the ice-box for a few hours and to examine it from time to time to ascertain whether hemolysis takes place on standing. If this should be the case to any marked extent, the antigen probably possesses hemolytic properties in itself and is then likewise undesirable. Formerly when simple alcoholic extracts were almost exclusively in use this was a not infrequent occurrence, but with the extracts prepared as described above it is uncommon. After the antigen has been tested in these various directions it should be kept in the dark and preferably in the ice-box. It will then not change its titer for a number of months, but should not be looked upon as a stable product. In my laboratory we test the titer about once a month, and have reason to urge this rule upon others.

2. *The Hemolytic Amboceptor*.—To prepare the hemolytic amboceptor a large rabbit is injected on two occasions, seven days apart, with the washed corpuscles corresponding to 30 c.c. of sheeps' blood, which must be obtained under aseptic precautions, and after removal of the serum by centrifugation, washed with at least three changes of sterile 0.9 per cent. salt solution. Care should be had each time, after packing down the corpuscles by centrifugation and pipetting off the washings, to stir up the corpuscles in the new portion of saline that is added. Finally, the corpuscles are suspended in such an amount of saline that the volume injected equals that of the full

blood which was originally used. From nine to eleven days later, according to the amboceptor content, which can be readily ascertained by a preliminary test of a few drops of blood, the animal is bled to death, the blood being collected under aseptic precautions. To this end it is convenient to use a test-tube which has been drawn out into a capillary near its closed end, at an angle of about 115 degrees. This is sealed, the open end closed with cotton, and the whole sterilized. After the animal has been anesthetized, the neck is shaved, scrubbed with soap and alcohol, and the carotid dissected out through a median incision. The tip of the capillary is broken off and the tube, moistened with sterile saline, introduced into the vessel, when the blood will rise into the collecting tube. The capillary is quickly sealed in a flame and the tube then placed on ice for the serum to separate out. Subsequently, the serum is pipetted off with a sterile pipette, heated for thirty minutes at 56° C., treated with carbolic acid to the extent of 0.5 per cent., and may then be kept in a dark colored bottle, well corked, on ice. Instead of doing this I find it more convenient to fill small glass beads with about 0.5 c.c. of the serum each, to seal these, and to keep them in an ice-box. The addition of carbolic acid is then not necessary.

The titer of the amboceptor should be at least such that 0.5 c.c. of a 1 to 2000 dilution (in 0.9 per cent. saline) will completely hemolyze 0.5 c.c. of a 5 per cent. emulsion of washed sheep corpuscles (see below), in the presence of 0.5 c.c. of a 1 in 10 dilution of guinea-pig complement (see below), within thirty minutes at 37° C. With the two injections of 30 c.c. of sheep blood each, one may at times obtain a serum which will still hemolyze this quantity of corpuscles in a dilution of 1 to 6000. At other times better results are obtained by giving the rabbit four or five injections of 5, 10, 15, and 20 c.c. of washed corpuscles, in succession, five days apart, the animal being killed when the desired titer has been reached.

Not every animal can be brought to a satisfactory titer, however, and during the winter months especially it is not unusual to find but one animal of perhaps a dozen that will furnish a satisfactory amboceptor.

Using one of the little beads just mentioned, I make up a 1 to 100 stock dilution which, when kept on ice, will usually retain its titer for many weeks, and is used to make up the higher dilutions on the days when these are wanted. It is best, however, to test it against

the complement anew at least once a week, as the activity of the complement varies considerably in different guinea-pigs. In the actual experiment, viz., in the study of the patient's serum, from two and one-half to three times the completely hemolyzing dose is used.

3. *The Washed Corpuscles.*—The necessary amount of sheep's blood is readily procured from a slaughtering house. If this is not available, a sheep may be kept near the laboratory and is bled from the ear as occasion demands. In the hemolytic experiment it is not essential to work aseptically. After separation of the serum the corpuscles are washed three times with saline, as mentioned above. At last all the fluid is carefully pipetted off; from the remaining corpuscles a 2.5 per cent. emulsion is prepared in saline, which corresponds to a 5 per cent. emulsion of the native blood.

We use the corpuscles only on the day on which they are procured and on the one following. They should be kept in the ice-box while not in use. If the supernatant fluid shows the least discoloration they should be discarded.¹

4. *The Complement.*—Guinea-pig serum is used as complement. As this is supposedly derived from disintegrating leukocytes, it is recommended to obtain the blood some hours before use. We usually kill the guinea-pig the evening before, by cutting the vessels of the neck, after anesthetizing the animal with ether. The blood is received in Petri dishes and is kept overnight on ice. The following morning the serum is pipetted off; if desired one can then place the clotted blood in centrifuge tubes and obtain still more serum by centrifugation. If it is not practical to kill the animal the evening before, this may be done in the morning of the day on which it is used; the blood is then placed on ice for two or three hours and the serum obtained by centrifugalizing the clot. Before use the serum is diluted 1 in 10. The unused portion of the concentrated serum may be kept frozen, for one or two days, but before further use it must be tested and adjusted to the hemolytic amboceptor as described. Very often it will be found to be inert. In my laboratory, we have set aside special days of the week for complement fixation work, and we then make no attempt to preserve any of the complement.

Where only a few specimens are to be examined at one time it

¹ For washing purposes, as well as for diluting the various reagents, it is essential to use chemically pure sodium chloride. Some of the tablets furnished by dealers will cause hemolysis in themselves.

is not necessary to kill the animal. A few cubic centimeters of blood can be obtained by puncturing the heart with an antitoxin syringe, under anesthesia. My own preference, however, is to kill the animal.

As has already been indicated, the complement, before use, whether fresh or not, must always be adjusted to the amboceptor.

5. *The Patient's Serum*.—It is generally recommended to secure blood from the patient as well as from the normal controls by venepuncture. This, however, is totally unnecessary. The required amount can be readily obtained from the ear. This is punctured with a small lancet or tenotomy knife, introducing the blade, at an angle, into the lobule and making a small sweep of the point of the blade without enlarging the skin incision, so as to cut a larger number of capillaries. Enough blood can then be milked out in about five minutes to fill a glass tube $1\frac{1}{2}$ to 2 inches long, and having an inside diameter of $\frac{1}{4}$ of an inch. The tube is corked and thus brought to the laboratory. The clot is then separated from the walls and the corpuscles packed down by centrifugation. The supernatant serum is pipetted off with Wright pipettes, placed in tubes similar to those in which the blood is collected and inactivated (complement destruction) by heating for thirty minutes at 56° C.; after this it is diluted 1 in 5, and is then ready for use.¹

A normal serum and a specimen from a known case of syphilis should always be available as controls.

It is recommended that all sera should be examined on the day on which they have been procured. This no doubt is a good rule, but I have found that fixing sera remain active for several weeks. It is thus perfectly feasible to send specimens from a distance, especially if the serum is separated from the corpuscles after bleeding the patient.

As human serum frequently contains amboceptors which are hemolytic for sheep corpuscles in the presence of complement, and as their amount is variable and at times not inconsiderable, a factor is here introduced into the experiment which could convert a positive into a negative result. For we must bear in mind that the activity of amboceptor and complement stand in an inverse proportion to one another, such that a very small amount of complement would

¹ If it should be desired to secure somewhat larger amounts of blood by venepuncture, the vacuum bulb recommended by Keidel will be found very convenient (see Jour. Amer. Med. Assoc., May 25, 1912, p. 1579).

be quite sufficient to effect a very considerable degree of hemolysis, if amboceptor were present in excess.

Some investigators, such as Noguchi, have accordingly recommended the use of an antihuman instead of an antisheep hemolytic system. I have found, however, that this is not only inconvenient, but also unnecessary, as the natural antisheep amboceptor can be readily removed by merely diluting the inactivated serum of the patient with five times its volume of the corpuscle emulsion, and incubating the mixture for thirty minutes in the water-bath at 37° C.; the corpuscles with the anchored "natural" antisheep amboceptor are then thrown down with the centrifuge, when the supernatant, now already diluted, serum is ready for use. This step is now carried out as a matter of routine in my own laboratories, and assures perfectly satisfactory results; with the use of the Noguchi antigen, and this modification the objectionable "Nachlösung" (continuing hemolysis at the end of the experiment) is no longer a source of error and hence of worry. The *test-tubes* which we use measure 4 inches in length by $\frac{3}{8}$ inch inside diameter.

METHOD.—When everything is in readiness the complement and amboceptor are adjusted to one another, using dilutions of 1 to 1000, 1 to 2000, 1 to 3000 up to 1 to 6000 of the amboceptor; 0.5 c.c. is our unit of measure, and we accordingly combine 0.5 of the various amboceptor dilutions with 0.5 c.c. of the complement (1 in 10) and 0.5 c.c. of the corpuscle emulsion (5 per cent.). The tubes are placed in the water-bath at 37° C., and are frequently shaken. At the expiration of thirty minutes the highest dilution is noted at which complete hemolysis occurs. The amboceptor dilution to be used in the actual experiment is then made $2\frac{1}{2}$ to 3 times as strong. Thus, if complete hemolysis occurred at 1 to 6000, we would use a 1 to 2500 or a 1 to 2000 dilution.

The antigen has been previously tested, as described. With human heart antigen, one can usually use a dilution of 1 to 10.

The titers of the various reagents having thus been ascertained, the experiment proper can now be carried out (tubes marked E), using 0.5 c.c. of the patient's serum (1 in 5)¹ combined with 0.5 c.c.

¹ The patient's serum has previously been freed of any natural antisheep amboceptors by diluting it (1 in 5) with the standard emulsion of sheep corpuscles and incubating for thirty minutes, after which the corpuscles are thrown down by centrifugation, when the supernatant fluid is pipetted off and can be immediately used in the experiment.

PLATE VI



A



B



C

Wassermann Reaction.

A, positive; *B*, partial; *C*, negative reaction.

Note undissolved blood corpuscles in *A*, partial hemolysis in *B*, and complete hemolysis in *C*.

of complement (1 in 10) and 0.5 c.c. of antigen (1 in 10). At the same time controls (tubes marked C) are prepared in which the antigen is left out, so that 0.5 of each serum is combined with 0.5 c.c. of complement and 0.5 c.c. of saline (in place of the antigen). The E and C tubes properly marked with the patient's numbers are placed in the water-bath for thirty minutes and then receive, each, 0.5 c.c. of the hemolytic amboceptor and 0.5 c.c. of the corpuscles. They are then returned and left for thirty minutes longer, the tubes being frequently shaken. After that some writers recommend that they be placed on ice and examined the next morning. I can see no advantage in this delay, and prefer to centrifugalize the tubes and read them at once. Strictly speaking it is not necessary to wait even thirty minutes if one places a tube containing antigen-complement-normal serum in the lot and breaks off the incubation as soon as this control is completely hemolyzed.

RESULTS.—Complete inhibition or absolute fixation is, of course, at once evident from the fact that the supernatant fluid (after centrifugation) is perfectly colorless, the corpuscles being all at the bottom. Partial fixation will show itself by a more or less colored supernatant fluid and the presence of a varying number of undissolved red cells at the bottom, while with complete hemolysis there is no sediment of red cells whatever. The results are accordingly noted as + + +, + +, +, \pm , and 0 (see Plate VI).

On the question of a well-marked fixation there can, of course, be no dispute, but with slight fixations errors are very apt to creep in. We would suggest that slight fixation be neglected and reexaminations made, especially in cases which are submitted for diagnosis.

The controls will usually be found hemolyzed completely, but at times, though rarely, sera are met with which fix more or less completely by themselves. In such cases it would, of course, not be warrantable to say that the reaction in the E tube was due to syphilis. What this independent inhibition means we do not know.

Regarding the *value of the Wassermann* reaction, both from the standpoint of diagnosis and in its bearing upon the question of treatment, we would emphasize that its neglect in a doubtful case from either point of view would constitute a grave *Kunstfehler*,¹ as

¹ An error of omission would approximately express the idea in our own language.

the Germans put it, of which, very fortunately, but few modern physicians are apt to be guilty.

Considered from the diagnostic standpoint a *well-pronounced positive* reaction may probably always be regarded as indicating the existence of syphilis, if we can rule out such diseases as frambesia, leprosy, sleeping sickness, and scarlatina. In malignant disease a certain degree of complement fixation may also be obtained, in a considerable number of cases, but I have not been able to convince myself that a triple plus (+++) reaction can ever be ascribed to the malignant process in itself. Partial reactions (+ or \pm), on the other hand, are here not infrequently met with and may even be seen in persons who neither show any present signs nor give any history of syphilis in the past. What these feeble reactions mean we do not know, but we are inclined to think that they may possibly be the expression of some inherited syphilitic taint, though we have but few data to support this belief. As the result of a fairly wide experience with the reaction we have come to the conclusion that from the diagnostic standpoint triple plus reactions *only* should be considered as positive evidence of syphilis, while the feebler grades, in individuals with an admitted history of the disease, may be regarded as indicating that the infection is probably limited to relatively small areas, from which an insignificant absorption of spirochetal substance is taking place, with a correspondingly limited formation of the lipoidophilic antibody. If the actual focus of infection should be sufficiently restricted it is, of course, conceivable that a negative reaction even might be obtained, and it is for this reason that a single negative reaction has a limited value only from the standpoint of diagnosis as well as of treatment. As pointed out in a previous chapter, however (see section on Salvarsan), it is frequently possible by the administration of a few large doses of mercury to evoke a positive reaction in individuals in whom the disease has almost been eradicated, whereby a larger number of spirochetes is destroyed at one time and a more intense stimulus given to antibody formation (*provocatory stimulation*). This possibility has not yet received the recognition which it deserves, but should be utilized in all doubtful cases, as well as in determining whether a continuance of treatment is desirable or not (see page 274).

In very early cases of syphilis, in which a sufficient length of time for the formation of antibodies has not yet elapsed, the result will,

of course, also be negative, but in these the diagnosis can usually be made by direct demonstration of the spirochete with the microscope.

With the limitations just set forth a diagnosis of syphilis can be reached by means of the Wassermann reaction in over 90 per cent. of the cases taken at random, the different types giving different values, as shown in the accompanying table, which is taken from Noguchi. The values given were obtained with the Noguchi system, *i. e.*, with an antihuman hemolytic system, but represent practically what the method furnishes which we have described above.

NOGUCHI SYSTEM; SYPHILIS, PARASYPHILIS, HEREDITARY SYPHILIS, AND SYPHILIS SUSPECTS

	Cases examined	+		-	±
		No.	Per cent.		
Primary syphilis	70	65	92.8	4	1
Secondary syphilis	197	190	96.0	5	2
Tertiary syphilis	177	159	89.9	16	2
Early latent syphilis	115	87	75.6	24	4
Late latent syphilis	150	119	79.3	27	4
Under prolonged treatment	39	4	10.2	32	3
Cerebral syphilis	5	3	60.0	1	1
Tabes	125	85	68.0	27	13
General paralysis	15	13	86.0	2	0
Hereditary syphilis	17	17	100.0	0	0
Syphilis	172	60	34.8	96	16
	1082	802		234	46

COMPARISON OF THE WASSERMANN AND NOGUCHI SYSTEMS

	Cases examined	Wassermann			Noguchi		
		+		-	+		-
		No.	Per ct.		No.	Per ct.	
Primary syphilis	23	17	73.9	6	20	86.9	3
Secondary syphilis	79	69	87.3	10	76	96.2	3
Hereditary syphilis	65	52	80.0	13	57	87.6	8
Early latent syphilis	27	13	48.0	14	18	66.6	9
Late latent syphilis	32	24	75.0	8	27	84.3	5
Tabes	18	8	44.0	10	13	72.2	5
	244	183		61	211		33

COMPARISON OF THE WASSERMANN AND NOGUCHI SYSTEMS (RESULTS
OBTAINED BY D. M. KAPLAN)

	Cases examined	Wassermann			Noguchi		
		+		-	+		-
		No.	Per ct.		No.	Per ct.	
Primary syphilis	138	122	90	16	134	97	4
Secondary syphilis	281	242	86	39	270	98	11
Tertiary syphilis	191	140	73	51	155	81	36
Latent syphilis	79	41	51	38	60	75	19
Hereditary syphilis	20	18	90	2	18	90	2
Tabes	205	125	60	80	134	65	71
General paresis	61	40	65	21	44	72	17
Cases for diagnosis	311	98	31	213	180	57	131
	1286	826	—	460	995	—	291

As regards the *relation of the Wassermann reaction to the treatment of syphilis with salvarsan* or salvarsan in combination with mercury, the majority of syphilographers are in accord in demanding that the treatment be continued until a permanently negative Wassermann is obtained and maintained (see section on Salvarsan). This standpoint is in accord with the view that the Wassermann reaction is a reaction of infection and not of immunity, and that the existence of infection may be inferred so long as the reaction is demonstrable.

The rapidity with which the reaction disappears under treatment is quite variable. I have thus obtained a persistingly negative result already after a single injection of salvarsan, while in other cases the salvarsan in itself, though given repeatedly, was not able to cause the reaction to disappear, whereas this promptly occurred, if mercurial treatment was instituted in addition. For further details of this order, however, I must refer the reader to special works.

In this connection it is interesting to note that Noguchi has recently compared the findings obtained with the Wassermann technique, *i. e.*, with the use of lipoid antigen, with the results which were obtained, when a pure culture of spirochetes was used as antigen. I append some of the more important conclusions to which these investigations gave rise: "(1) The Wassermann reaction is caused by lipotropic substances, but not by the antibodies which combine specifically with the pallida antigen; (2) the fixation produced by the culture pallida antigen with certain syphilitic sera is caused by the specific antibodies contained in the latter and may constitute a specific diagnostic method for syphilis; (3) the fixation

caused by a syphilitic testicular extract behaves like the culture pallida extract in the majority of cases, but when the sera (syphilitic or leprous) contain abundant lipotropic substances, it may give a Wassermann reaction as well, which is not the case with the culture pallida antigen; and, finally, (4) in the serum of rabbits with active syphilitic orchitis there is no indication of the presence of a sufficient amount of the antibodies for the pallida antigen, although it gives a strong Wassermann reaction. It remains to be seen when and under what conditions the specific antibodies for the pallida will most abundantly be formed in syphilitic patients. At all events it is rather remarkable that the amount of the antibodies detectable by the pallida antigen in these cases was so small as compared with certain other infectious diseases, in this respect. It is not improbable that those who come under our care belong to a class of individuals with comparatively less resistance to the pallida and are incapable of producing sufficient antibodies, while there are many who respond to the infection with more vigorous formation of the antibodies and reduce the infection to a harmless latency or even destroy the pallida completely. This latter class of infected persons do not, of course, frequent our clinics. If this is the case, it would be of immense prognostic importance to check a patient from the beginning of infection by the complement fixation test with the pallida antigen, thereby determining the resistance of the patient against the disease.

"We have in the Wassermann reaction a fair measure of activity of the infecting agent, and now we will have in the pallida fixation reaction a gauge for the defensive activity of the infected host."

While the principle of complement fixation has thus far found its widest field of practical application in the diagnosis of syphilis, in the form of the Wassermann reaction, as just described, there is reason for thinking that the *diagnosis of latent gonococcus infections* also will ere long be possible upon the same experimental lines. Excellent results have already been reported from different sources.

Aside from this possibility the same principle may also be utilized in legal medicine when the question arises whether a certain blood-stain is of human origin or not. In such a case the material in question is brought into solution and is then tested as antigen against an active antihuman precipitating serum (see Precipitin Reaction, below), which has been obtained by immunizing rabbits with human

blood serum, this antiserum taking the place of the antibody of the Wassermann reaction. If, then, the suspected substance contains human albumins these will react with the corresponding precipitin of the antiserum, with the result that any complement that may simultaneously be present is bound to a greater or less extent exactly as in the case of the Wassermann reaction.

PRECIPITIN REACTIONS

Following the demonstration by Tschistovitch and Bordet (1899) that not only vegetable albumins but animal albumins also are capable of giving rise to precipitin formation, when injected into animals of an alien species, Uhlenhuth especially drew attention to the remarkable specificity of the reaction when applied to the study of the blood of different animals. He thus laid the foundation of the modern *biological blood test*, which is now recognized as proper evidence regarding the origin of blood-stains, in the courts of practically all civilized countries.

Aside from these more practical bearings the precipitin reaction has attracted a great deal of attention owing to the unexpected light which it has thrown upon the biological relationship existing between different animals. For it has been shown that while the precipitins which can be produced in a rabbit, for example, by the injection of the serum of a horse and which naturally will react with the latter, likewise do so with the serum of the donkey and the tapir. An antidog serum will similarly react with the serum of the fox, antichickens serum with pigeon serum, antigoat serum with sheep and bovine serum, antihuman serum with the serum of apes, etc.

These *group reactions* are readily explained if we assume the existence in the antigenic sera of "*partial*" *precipitinogens*, i. e., of precipitinogenic molecular complexes which are peculiar to a special species, besides others which are common to a whole group of species, all of which will naturally give rise to corresponding "*partial*" *precipitins*, in a manner quite analogous to the formation of "*partial*" agglutinins (which see). That such partial precipitins actually exist in an antiserum may be shown by treating antihuman serum with monkey serum when the antimonkey precipitin will

cause the formation of a corresponding precipitate. If this is then removed by centrifugation (quantitative relations being, of course, duly considered) the remaining serum may be shown to have retained its precipitin for human serum, while that for monkey serum has disappeared. That antihuman serum, moreover, should possess a larger quantity of antihuman than of antimonkey precipitin would naturally suggest itself and can be demonstrated by suitable methods.

The technique which is involved in these various examinations may be suitably described in connection with its application to the medico-legal blood test, according to Uhlenhuth.

The Biological Blood Test.—As the legal question at issue is usually whether or not a certain blood-stain is of human origin, it is ordinarily only necessary to examine the material in question in reference to its behavior toward an antihuman serum. If, on the other hand, the antihuman investigation has shown that the material was not of human origin, and it is desired to ascertain from what animal species the blood was derived, corresponding sera must, of course, also be available.

PREPARATION OF THE ANTISERA.—The antisera in question are usually obtained from rabbits after injection with either human serum, pig serum, or bovine serum, etc., as the case may be. The injections are given intraperitoneally or intravenously at intervals of five or six days, using 10, 8, and 5 c.c. respectively in the first instance, and 5, 3, and 2 c.c. if the latter method is preferred. It is always best to inject several rabbits at the same time, especially since not every animal furnishes a serum with a sufficiently high titer. According to Uhlenhuth this should be such that 0.1 c.c. of the antiserum shall produce a distinct turbidity either instantaneously or at most after one to two minutes, when added to 1 c.c. of a 1 to 1000 dilution of the corresponding *antigenic* serum. Added to 1 c.c. of a 1 to 10,000 and 1 to 20,000 dilution a turbidity should be discernible after three and five minutes respectively, while a control specimen, containing only 0.85 per cent. saline (the diluent in question) and 0.1 c.c. of the antiserum must, of course, remain clear. The reaction is best observed by holding the tubes (without shaking) against a dark background, when it will be seen that the turbidity first appears as a faint opalescence at the bottom of the tubes, but in the course of five minutes extends throughout the specimen, becoming increasingly denser and ultimately settling to the bottom as a precipitate.

The desired titer is frequently obtained already on the sixth day following the last injection. If an examination of a test specimen taken from the ear does not indicate the desired strength at this time, it may be necessary to give a fourth, a fifth, and even a sixth injection, but it may also happen that the particular animal cannot be brought to the titer that is necessary, with any number of injections. If, however, the examination shows that the serum can be used, the animal is bled to death, the serum separated by centrifugation, cleared by passing through a Berkefeld filter, and finally stored in little glass beads or ampoules in portions of 1 c.c. each. No preservative is added, and it is accordingly necessary throughout to observe aseptic precautions.

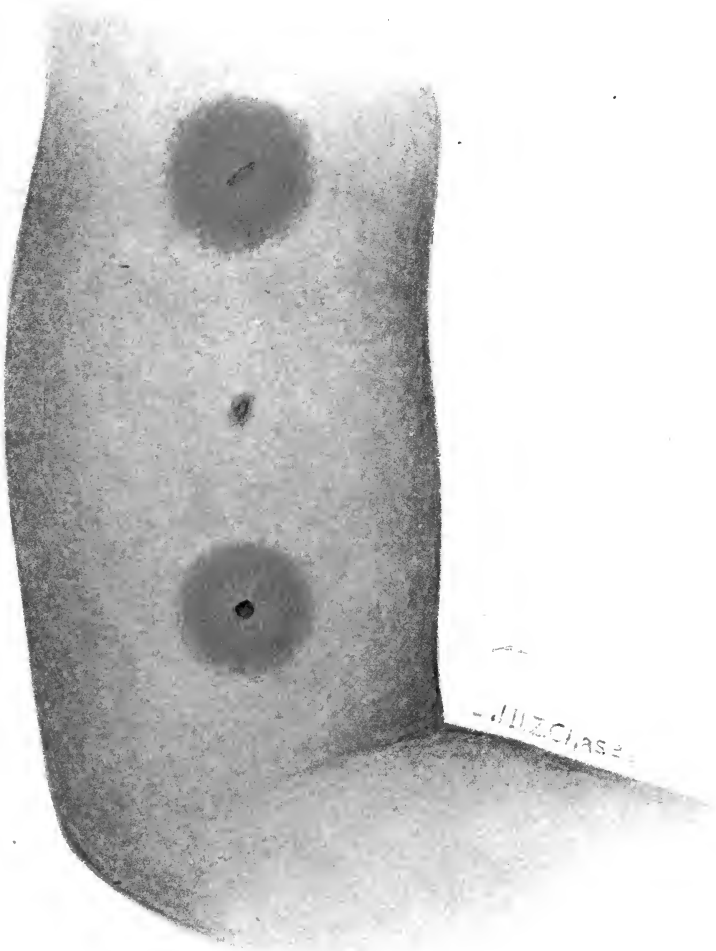
All examinations are conducted in little test-tubes, such as those used in the Wassermann work, which must, of course, be scrupulously clean. Or, if very small amounts of material only are available for the examination, this is conducted in glass capillaries. The turbidity then develops at the zone of contact between the two fluids, and may be advantageously observed with the aid of a magnifying glass.

PREPARATION OF THE SUSPECTED MATERIAL.—This is brought into solution with the aid of 0.85 per cent. saline, and is then further diluted to such a degree that on boiling a small amount (1 c.c.) with a drop of 25 per cent. nitric acid a slight opalescence develops. This would correspond to a 1 to 1000 dilution of the blood in its original state and represents the minimal degree of dilution (*i. e.*, the maximal concentration) with which the actual test should be made.

The solution of the suspected material should, of course, also be perfectly clear, to which end it may be necessary to pass it through a Berkefeld filter, or, if the quantity be small, through a Silberschmidt microfilter.

THE EXAMINATION PROPER.—Six tubes are placed in a suitable rack and labelled I, II, III, IV, V, and VI. Tube I and II receive 1 c.c. of the solution under investigation, III and IV 1 c.c. of two control solutions made up from dried *animal* blood, *i. e.*, from blood which does not correspond to the antiserum that is used, *e. g.*, cat or dog blood, if the antiserum is antihuman in character, and which has likewise been diluted so as to correspond to a 1 to 1000 solution (see preceding section), V 1 c.c. of sterile 0.85 per cent. saline, and VI 1 c.c. of a 1 to 1000 solution of blood (made up of dried material).

PLATE VII



Cutaneous Tuberculin Reaction of v. Pirquet.
(Taken from Hamill.)

corresponding to the antiserum in question, *i. e.*, of human blood, if the antiserum was antihuman in character. To each tube, with the exception of tube II (which is treated with 0.1 c.c. of *normal* rabbit serum), 0.1 c.c. of the corresponding antiserum is then added in such a manner that the serum flows down the side of the tube and does not drop directly into the fluid below. The tubes are now allowed to stand at room temperature and without shaking for twenty minutes, when the final reading is made. If the result is positive, *i. e.*, if the suspected material was of human origin, precipitation will occur in tubes I and VI, while II, III, IV, and V remain clear.

With this method reliable results can be obtained, so long as the material under examination contains albumins which are still capable of undergoing solution, even though they be present only in traces. Uhlenhuth and Beumer thus mention that they obtained positive results with blood which had undergone putrefaction and had been left exposed to the air for two years, as well as with dried blood-stains which were more than fifty years old.

FERMENT REACTIONS

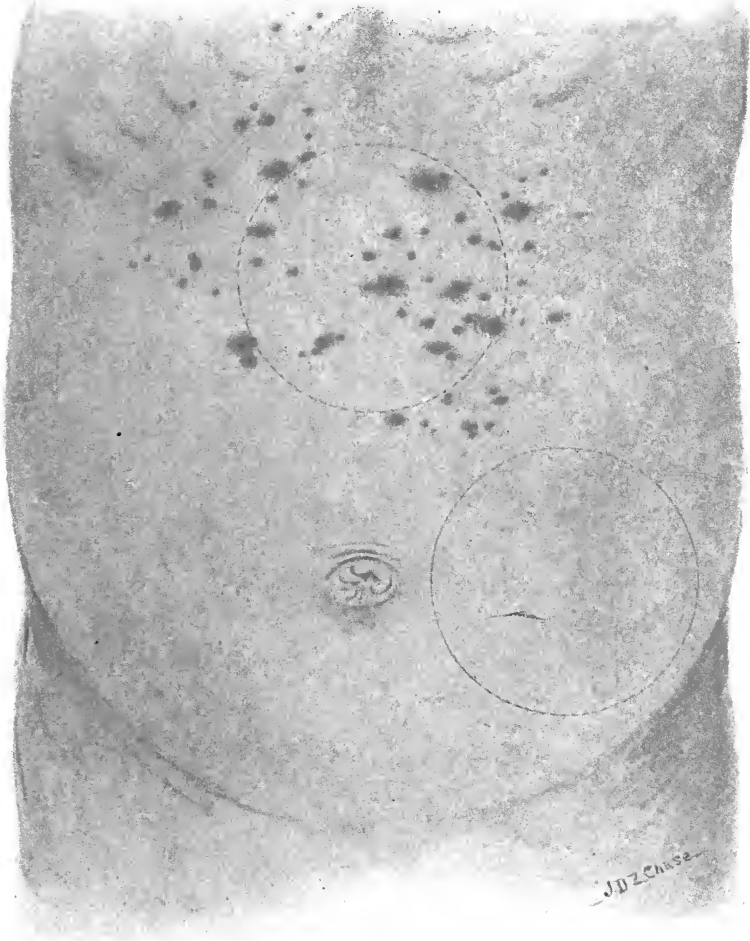
The Pregnancy Test of Abderhalden.—This test is primarily based upon the observation that the parenteral introduction of complex foodstuffs of alien origin either leads to the appearance in the blood serum of reaction products *de novo* which are capable of causing the change of such bodies, or it increases the quantity of corresponding products which may normally be present. Rabbit serum has thus no digestive properties for silk peptone, while the serum of an animal that has been previously injected with such material is capable of effecting its cleavage. Similarly we find that normal serum is incapable of bringing about the cleavage of cane sugar, while the serum of an animal that has previously been injected with the carbohydrate in question does this quite readily. Most extensive investigations along these lines convinced Abderhalden that such a reaction on the part of the treated animal is of invariable occurrence, and in a general way this corresponds to our views regarding antibody formation, as set forth in the first part of this volume. Without entering into the question regarding the possible identity of the antibodies in the sense of Ehrlich and the digestive reaction products with

which we have just become acquainted, the thought naturally suggests itself that any component of the body may in the end be viewed as alien to other parts of the body, if it is placed in surroundings which are in reality alien to that particular component. It is thus quite conceivable that the presence in the circulation of some of the body's own cells might give rise to similar reactions providing that the cells in question are really foreign to the interior of the bloodvessels, *i. e.*, to the blood plasma or the blood cells. As this takes place not only under various pathological conditions, but even during pregnancy, where chorion cells have been shown to enter the circulation, it was natural to put the question to the experimental test. As a result Abderhalden announced that he succeeded in demonstrating that the blood serum of pregnant animals has acquired the power of causing the cleavage of placental peptone and placental proteins.

While his experiments were originally conducted by bringing together the serum of the pregnant animal and the placental peptone in the tube of the polarimeter, when a gradual change in the degree of rotation could be noted as the cleavage took place, he subsequently discovered that the same reaction can be demonstrated by placing the placental tissue together with the blood serum of the individual in a dialyzing tube and then testing the dialyzate for biuret. To this end the following procedure is recommended.

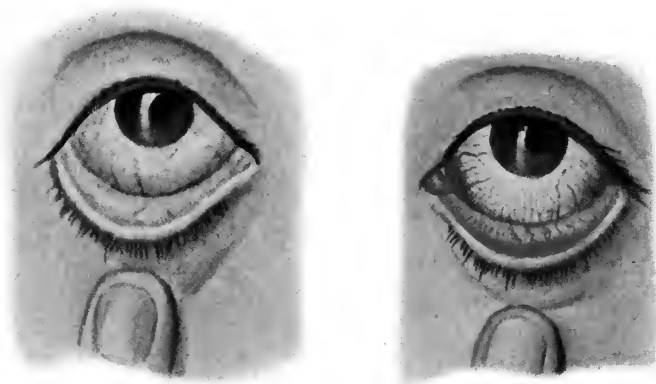
Abderhalden's Test.—*Preparation of the Antigen.*—A fresh placenta is stripped of its membrane, cut into small pieces and washed free from blood by kneading the material in running water. It is then placed in boiling water which has been slightly acidified with acetic acid (2 drops of the glacial acid to a liter of water), and the boiling continued for five minutes. The supernatant fluid is decanted and replaced by a corresponding quantity of water. The boiling is continued for five minutes longer, and the water then tested for biuret (see below). So long as a positive reaction is obtained the tissue is boiled with new portions of water, until finally it is placed in a bottle (together with the last portion of water) and covered with a layer of toluol. In this form it keeps practically indefinitely. It is advisable nevertheless to assure one's self from time to time that the biuret reaction is still negative; if this should not be the case the material is boiled with new portions of water until the desired end has been attained.

PLATE VIII



Cutaneous Tuberculin Reaction of Moro.
(Taken from Hamill.)

PLATE IX



Tuberculin Ophthalmo-reaction. (Taken from Citron.)

Diffusion Tubes.—For purposes of dialysis Abderhalden recommends parchment tubes prepared by Schleicher and Schüll (No. 597). These may be kept in water covered with toluol and should never be used while dry or without being previously tested for their tightness. To this end a small quantity of normal serum is dialyzed under toluol against distilled water (as described below) for twenty-four hours, when the dialyzate is tested for biuret. Should the reaction be positive it is clear that the tube is not tight enough. Judd found in my laboratory that there are only about six tubes in twenty-five (of the number which Abderhalden recommends) which are serviceable in this respect, the majority permitting the diffusion of proteins which will likewise react with the new triketo reagent, which is usually employed in testing for biuret. On the other hand it is well to test the tubes with a little Witte peptone solution, so as to make sure that they are not too tight. This, however, seems to be rare.

After use the tubes should be washed in running water, until the surrounding liquid no longer gives the biuret reaction. They are then kept in water under toluol until they are needed again, or, as Judd recommends, they may be suspended in distilled water in the corresponding cylinders, the latter stoppered with cotton, and sterilized by steam, when they are ready for use.

The Biuret Test.—While fairly satisfactory results may be obtained with the old copper sulphate test, using 5 c.c. of 33 per cent. caustic soda solution for 10 c.c. of the dialyzate, mixing well and superimposing a layer measuring 0.25 to 0.5 cm. of an 0.25 per cent. solution of copper sulphate, the triketohydrinden hydrate (Ninhydrin) test is in many respects to be preferred and much more sensitive. For this reason it would not be advisable to use the latter test in the final examination if the less sensitive copper sulphate reaction only has been used in testing the placental tissue for biuret. The triketo reagent is a white crystalline substance, which is employed in a 1 per cent. aqueous solution. On adding 0.2 c.c. of this to a solution of peptone a violet color develops on boiling. (This may be tried with a very dilute solution of Witte peptone.)

Preparation of the Serum.—The blood should be obtained under aseptic precautions and examined as fresh as possible. From 5 to 10 c.c. are best withdrawn with a syringe from one of the veins at the bend of the elbow. The serum is separated from the corpuscles by centrifugation in the usual manner.

The Test Proper.—From 0.5 to 1 gram of the placental tissue together with 2 or 3 c.c. of the serum is placed in one of the diffusion tubes (A) that has previously been adjusted in a small cylinder containing about 15 to 20 c.c. of distilled water, and both the serum and the outer fluid covered with a layer of toluol. A second tube (B) is similarly arranged, but receives serum only, and no placental tissue, while in a third tube (C) placental tissue is placed together with 2 or 3 c.c. of the water in which it was kept, all the tubes and cylinders being guarded against putrefactive changes by the addition of a liberal quantity of toluol. The three cylinders are then placed in the incubator and left for twelve to twenty-four hours, when the dialyzate (10 c.c.) is tested for biuret as described. If the test is satisfactorily positive tube B and C should give a negative reaction and A a positive reaction.

Results.—Abderhalden claims that a positive reaction may be obtained in every case of pregnancy even as early as the first month and that the reaction persists until about the end of the first week of the puerperal period. Corresponding results were reached in pregnant cows, dogs, guinea-pigs, and rabbits (when a homologous placental antigen must of course be used). Other observers who have repeated Abderhalden's work have obtained corresponding results and it would seem that the method may be regarded as one whose value is already beyond dispute.

That the same principle may be of use in the diagnosis of pathological conditions also seems quite possible and Abderhalden himself has already pointed out that cancer would suggest itself as a profitable field for investigation along these lines.

ALLERGIC REACTIONS

By the term allergic reaction in the clinical sense we understand the specific symptomatic response on the part of the infected and hence sensitized organism to the parenteral reintroduction of the corresponding antigen. Among the infectious diseases such reactions have been notably studied in connection with tuberculosis, but are evidently destined to play an important role in the diagnosis of other diseases as well. In the present work we shall confine our attention to the tubercular test and the luetin reaction in their relation to the diagnosis of tuberculosis and syphilis respectively.

The Tuberculin Test.—It will be recalled that the tubercular guinea-pig responds quite differently to the introduction of living tubercle bacilli than does the normal animal. For whereas in the latter a local reaction occurs only after from ten to fourteen days, definite changes can be detected in the former already within twenty-four to forty-eight hours. But while in the primarily non-tubercular animal the local lesion then remains active to the end, local recovery occurs in the reinjected tubercular pig. If an emulsion of dead organisms (tuberculin) be used instead, as much as 0.5 gram may be injected, intraperitoneally even, in the case of the normal animal without producing any deleterious results, while similar treatment of the tubercular guinea-pig would lead to a fatal ending. If the injection is made subcutaneously, and the dose is chosen sufficiently small as not to kill, a severe local reaction will result, as in the first instance, where living organisms were used, and incidentally it will be observed that in the tubercular in contradistinction to the non-tubercular animal, temporarily at least, certain general symptoms of illness develop, of which a rise in temperature is the most striking and the most constant. Evidently the primary inoculation, while increasing the resistance of the animal to subsequent infection with the organism in question (immunity), has called forth a general, increased susceptibility to the action of its products of disintegration (anaphylaxis). According to v. Pirquet this difference in response is readily accounted for, if we remember that the parenteral introduction of foreign proteins (in the present instance of bacterial proteins) leads to the formation of corresponding antibodies, and that as a consequence of the interaction between the two groups of substances, in the presence of complement, toxic bodies (anaphylatoxins) are formed which may then produce symptoms of variable nature, according to the character of the tissues which are susceptible to their action.

In man results have been obtained which are perfectly analogous to those observed in the guinea-pig. The subcutaneous injection of the non-tubercular individual with small doses of tuberculin will thus produce no deleterious consequences whatever, while in the tubercular subject the same dose causes the well-known *general response* by headache, muscle pains, and fever, besides the *local inflammatory reaction* at the site of the injection. In cases where the tubercular lesion is superficially located and can be directly

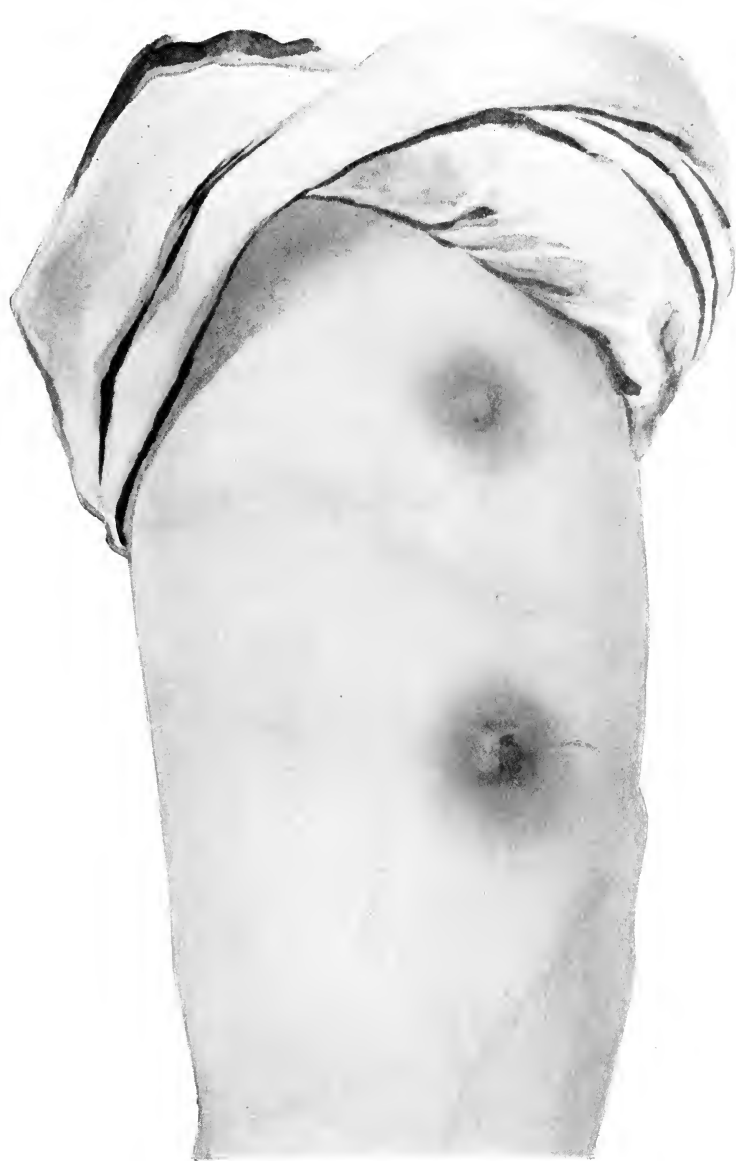
observed the development of increased redness and swelling, moreover, give evidence of a direct effect upon the seat of infection. The same is shown by the increase in the number of the rales and in the number of bacilli in the sputum,* if the injection is given in a case of pulmonary tuberculosis (*focal reactions*).

If, on the other hand, the tuberculin is administered in such a manner that active resorption does not occur, local reactions only will be observed, and in tissues, it should be remembered, which are not tubercular in themselves. Following a cutaneous inoculation with tuberculin an inflammatory papule thus appears, no matter at what point the injection is made (v. Pirquet reaction), instillation into the conjunctival sac gives rise to an intense conjunctivitis (Calmette reaction); innunction with a tubercular salve calls forth a local dermatitis (Moro reaction), etc.

Owing to this remarkable hypersusceptibility to tuberculin on the part of the tubercular subject, the principle in question has been extensively utilized for diagnostic purposes, and it may not be out of place to briefly describe the most important methods which have been advocated for this purpose.

The Tuberculin Test According to Koch (Subcutaneous Method).—The material which is employed to this end is the old tuberculin of Koch. Of this the patient receives from 0.1 to 1 milligram, according to the condition of his general health. In feeble individuals it is best to start with 0.1 milligram, while more robust persons may take 1 milligram. The injections are conveniently given in the back, below the angle of the scapula, and best during the early forenoon hours. To wait until the evening is not advisable, as the reaction may occur already after six hours and might accordingly be overlooked during the night. If no elevation of temperature occurs after the first dose the quantity is doubled in forty-eight hours, and so on until a dose of 10 milligrams, or in individuals of feeble constitution, of 5 milligrams is reached. This Koch regards as the limit, beyond which a reaction cannot be considered as specific. Should elevation of temperature follow any one of the injections, even though amounting to but three-tenths of a degree (C.), the next dose should be of the same size, but it is not to be given until the temperature has returned to normal. It will often be found, then, that the second reaction is more marked than the first. Such an occurrence Koch regards as particularly characteristic, and, indeed, as an infallible indication of the existence of tuberculosis.

PLATE X



Cutaneous Luetin Reaction, Severe. (Taken
from Noguchi.)



PLATE XI



Left



Right

Cutaneous Luetin Reaction, Moderate. (Taken from Noguchi.)



Before beginning it is, of course, desirable to observe the temperature of the patient for a while, and not to inject until it has been found below 37.3° C. for a day or two.¹ The reaction is regarded as positive if the temperature reaches a point that is at least 0.5° C. above the highest noted before the injection.

If small doses have been used the rise usually begins after ten to sixteen hours, while with the larger doses it may occur after six to eight hours. There is usually a slight chill which is accompanied by headache and pains in the muscles, nausea, palpitation of the heart, etc. An hour or two after the injection already there may also be evidence of an inflammatory reaction at the point of inoculation (redness and tenderness). At the expiration of about ten hours there is marked infiltration at this point which may persist for two to six days before resorption has taken place. After reaching its highest point the temperature usually drops within a few hours, so that normal relations are again restored at the expiration of twenty-four to forty-eight hours following the injection. The patient may experience a certain degree of lassitude yet for two or three days and possibly have an increased secretion of sputum, but is then restored to the same condition as before the examination.

A positive reaction, of course, only means that the patient has a tubercular focus somewhere in his body, but does not in itself indicate whether this is active or not. This point must be decided by the history, the clinical findings, etc.

Regarding the *constancy of the reaction* in cases of proved tuberculosis, in suspected cases and in supposedly non-tubercular individuals the accompanying table will furnish the desired information:

Pulmonary tuberculosis.	90.0 to 100.0 per cent.
Suspected cases	92.1 per cent.
Non-suspected cases	56.1 per cent.

The high percentage of positive findings in non-suspected cases is readily explained, if we bear in mind how common a latent, inactive tuberculosis actually is.

As to *indications* and *contra-indications* it will suffice to state that the test may be made in all suspected cases unless heart lesions, diabetes, nephritis, or pregnancy exist, or unless laryngeal tuberculosis is suspected.

¹ The temperature should be taken every three hours.

To illustrate the general safety of the procedure, providing that the rules of dosage given above are implicitly followed, we would point out that Löwenstein did not meet with any serious symptoms or a single death in a series of 20,000 single injections which were made under his direction.

The Tuberculin Test According to v. Pirquet (Cutaneous Method).—The inner surface of the forearm is cleansed with ether, then two drops of the concentrated old tuberculin of Koch are placed about 10 cm. apart. With a special instrument, which v. Pirquet terms an "Impfbohrer" (vaccination gimlet), and which is essentially an exceedingly fine chisel with a platinum iridium point that can be sterilized in a flame, a small abrasion is first produced midway between the two drops. To this end the instrument is pressed against the skin and rotated, sufficient force being employed to produce a definite abrasion, without, however, causing any bleeding. A similar scarification is then made through each one of the two drops of tuberculin. A tiny bit of sterile absorbent cotton is now laid across each drop so as to prevent it from flowing away. After five minutes this is removed. A dressing is not used. Should examination at the expiration of twenty-four to forty-eight hours not reveal the existence of a distinct brown scab measuring about 1 mm. in diameter, both at the point of inoculation as well as at that of control, the abrasion has been too slight, and the test must be repeated.

The appearance of a positive reaction when fully developed is well shown in Plate VII, and contrasts markedly with that of the control. If the abrasions are examined at frequent intervals it will be observed that a small wheal appears within a few minutes both at the control and the test point which soon becomes surrounded by a pink halo. This disappears after a few hours, leaving a small red area, in the centre of which a tiny scab begins to form. At the control point the redness is still discernible after twenty-four hours, but then fades away. At the test-point, in positive cases, the red area begins to increase in size after a period of time which varies between three hours and several days. Coincidentally the inflammatory area becomes elevated (papular) and develops rapidly in size. At the end of forty-eight hours the reaction has usually reached its height. At this time the diameter of the "papule" will average about 10 mm., but it may be much larger—up to 30 mm., the size, *cæteris paribus*, depending upon the quantity of tuberculin

which has been absorbed. The centre of the papule is sometimes pale, like an urticarial wheal. The surface otherwise is frequently finely vesiculated; pustulation, however, never occurs. While ordinarily the entire area is intensely hyperemic, nearly colorless papules are sometimes seen in very advanced cases of tuberculosis, at a time when the power of reaction on the part of the individual has almost disappeared (cachectic reactions). The hyperemic area is usually limited to the papule itself, but occasionally extends beyond, forming an areola, which strongly reminds one of what is seen in cases of vaccination.

After having reached its height the exudation gradually subsides. The swelling disappears in from five to eight days, but the pigmentation which then develops frequently remains visible for a number of weeks.

Exceptionally the reaction does not begin to develop until after twenty-four hours following the inoculation. Such a delayed response v. Pirquet speaks of as a *torpid reaction*. This is notably seen in individuals who show no clinical evidence of tuberculosis, and is the more frequent the older the patient.

The great advantage of v. Pirquet's method as compared with the older subcutaneous method of Koch is, of course, its simplicity, and the fact that undesirable systemic effects hardly ever occur, providing that the abrasion has been made *lege artis*, and that opportunity for undue absorption has not been afforded. As in the case of the subcutaneous method, however, a positive reaction merely denotes the presence of a tubercular focus somewhere in the body, which need not be active, however, and the diagnostic value of the method is hence limited to the same extent and even more. The greatest sphere of usefulness indeed seems to lie in its application to the diagnosis of tuberculosis in very young children. From a study of 757 children in which the test had been applied by v. Pirquet and in which the results were compared with the clinical findings, it appears that of the clinically tubercular cases 87 per cent. gave the reaction, while this was also found in 20 per cent. of the non-suspected cases. The negatively reacting tubercular cases, v. Pirquet points out, were almost exclusively cachectic or in the last stages of miliary tuberculosis.

As the result of a study of 124 children which had come to autopsy and in which the test had been made, v. Pirquet concludes that a

positive cutaneous reaction is never observed in the absence of a tubercular lesion; that a negative reaction ordinarily indicates freedom from tuberculosis, but that such a result may also be obtained in the last stages of the disease. As a positive reaction may be expected in over 90 per cent. of all individuals after the fourteenth year, it is clear, however, that the diagnostic significance of the reaction is then practically *nil*. As 35 per cent. of all children, moreover, give a positive cutaneous reaction between the ages of six and ten already, it is evident that even at this age its diagnostic value is limited.

The Tuberculin Test According to Calmette (Conjunctival Method).—While Calmette advocates the use of a tuberculin which essentially contains the alcohol-insoluble constituents of bovine tubercle bacilli, made up into a $\frac{1}{2}$ per cent. aqueous solution, one may also employ a 5 per cent. solution of the old tuberculin of Koch. One or two drops of either solution are placed upon the conjunctiva of one eye near its inner canthus, when the lids are held together for about a minute. In the normal individual slight redness may then develop and persist for a few hours, after which it disappears. In the tubercular subject, on the other hand, marked hyperemia occurs after three to six hours (more rarely after twelve to twenty-four hours); this principally affects the lower lid, the lower portion of the eyeball, the caruncle and the semilunar fold (see Plate VIII). At the same time there is some swelling and secretion, which in severe reactions becomes mucopurulent.

The height of the reaction is reached after ten to twelve hours, after which the inflammatory manifestations usually disappear and there is a return to the normal.

While in most cases no unduly severe reactions occur, such have nevertheless been noted in isolated cases, and a number of observers look upon the method in its original form as dangerous and not justifiable. Eppenstein accordingly recommends successive tests with solutions of increasing strength, and the use of both eyes alternately, beginning in adults with a 1 per cent. solution of the old tuberculin, and then increasing to a 2 per cent. and finally to a 4 per cent. solution, while in children a $\frac{1}{2}$ per cent. solution is used as the starting dose.

The existence of any disease of the eye would, of course, constitute a *contra-indication* to the method in question.

As regards the clinical value of the Calmette reaction, as com-

pared with the cutaneous reaction of v. Pirquet, it appears from an analysis of 2974 examinations collected by Petit that 94.3 per cent. of clinically tubercular cases showed the reaction, while among non-tubercular individuals only 18.4 per cent. reacted. The eye reaction would thus seem to be more useful from the diagnostic standpoint, and it is to be hoped that it may yet be improved to such a degree that dangerous reactions may with certainty be avoided.

As in the case of the v. Pirquet reaction, systemic and focal symptoms do not occur.

The Tuberculin Test According to Moro (Dermo-reaction).—Moro has shown that a skin reaction may be obtained in tubercular individuals after inoculation with a salve composed of equal parts of the old tuberculin of Koch and of lanolin. To this end a small amount of the salve (about the size of a pea) is for a minute rubbed into an area of the skin measuring not more than 5 cm. in diameter. The best district for this purpose is the skin just below the sternum or in the vicinity of the nipple. After drying for about ten minutes the patient may dress, no special covering being required. After twenty-four to forty-eight hours a dermatitis then develops which is characterized by the appearance of miliary nodules of variable size and number, which occur either singly or confluent. At the same time there is a more or less extensive general redness of the affected area, accompanied by a certain amount of itching (see Plate IX).

Regarding the clinical value of the method our knowledge is as yet too meager to warrant its general recommendation. v. Pirquet states that he has been able to obtain positive results only in highly susceptible individuals, but suggests that it may be tried, if for any reason the cutaneous or the eye reaction cannot be employed.

THE LUETIN REACTION

While a number of different investigators had previously attempted a skin reaction diagnosis in connection with syphilis, satisfactory results could hardly be expected so long as the successful cultivation of the corresponding spirochete in pure culture had not been accomplished. The solution of the latter problem we owe to the painstaking work of Noguchi, and to the same investigator belongs the credit

of having first prepared an antigen with which a specific syphilitic reaction may be obtained in a large percentage of infected individuals.

Preparation of the Antigen.—Pure placental ascites agar cultures of the pallida are ground up in a mortar with placental ascites bouillon cultures of the organisms until a fairly thin emulsion is obtained. This is sterilized for one hour at 60° C. and treated with tricresol to the extent of 0.5 per cent. The resultant product Noguchi has termed *luetin*. After being tested for its sterility it is ready for use. A similar preparation is made from sterile culture material and constitutes the *control fluid*.

Injection of the Patient.—Before using, the contents of the luetin bottle, which must be kept in the ice-box and frequently examined for its sterility, should be thoroughly shaken, so as to bring about an even suspension of the spirochetes. With a sterile pipette equal parts of the luetin and sterile saline are then placed in a tuberculin syringe and 0.07 c.c. injected in the case of an adult and 0.05 c.c. in infants. The injections are made *intracutaneously*, the left arm being chosen for the luetin and the right arm for the control fluid, of which a corresponding amount, likewise diluted with saline, is used. Separate syringes are kept for the luetin and the control fluid. Noguchi, moreover, advises that each arm be injected at two points, about 5 cm. apart. Considering the severity of some of the reactions, however, I should personally advise single injections.

Reactions.—While in non-syphilitic individuals the effect of the luetin and the control injection is identical and merely represents a slight traumatic reaction, which recedes within forty-eight hours and leaves no induration, there may be a marked difference between the two sides in syphilitic persons. Noguchi here distinguishes three types of reaction at the points where the luetin has been injected. In the first or *papular form* “a large, raised, reddish, indurated papule, usually from 5 to 10 mm. in diameter, makes its appearance in twenty-four to forty-eight hours. The papule may be surrounded by a diffuse zone of redness and show marked telangiectasis. The dimensions and the degree of induration slowly increase during the following three or four days, after which the inflammatory processes begin to recede. The color of the papule gradually becomes dark bluish red. The induration disappears within one week, except in certain instances, in which a trace of the reaction may persist for a longer period. This latter effect is usually seen among patients with

secondary syphilis under regular mercurial treatment in whom there are no manifest lesions at the time of making the skin test. Patients with congenital syphilis also show this reaction in the early period of life." In the second *pustular form* "the beginning and course of the reaction resemble the papular form until about the fourth day, when the inflammatory processes commence to progress. The surface of the indurated, round papule becomes mildly edematous, and multiple miliary vesicles occasionally form. At the same time a beginning central softening of the papule can be seen. Within the next twenty-four hours the papule changes into a vesicle, filled at first with a semi-opaque serum that later becomes definitely purulent. Soon after this the pustule ruptures spontaneously or after slight friction or pressure. The margin of the broken pustule remains indurated, while the defect caused by the escape of the pustular contents becomes quickly covered by a crust that falls off within a few days. About this time the induration usually disappears, leaving almost no scar after healing. There is a wide range of variation in the degree of intensity of the reaction described in different cases, as some show rather small pustules, while in others the pustule is much larger. This reaction was found almost constant in patients with tertiary or late hereditary syphilis" (see Plate X).

In the third or *torpid form*, which was only noted in rare instances, "the injection sites fade away to almost invisible points within three or four days, so that they may be passed over as negative reactions. But sometimes these spots suddenly light up again after ten days, or even longer, and progress to small pustular formation. The course of this pustule is similar to that described for the preceding form.

"This form of reaction has been observed in a case of primary syphilis, in one of hereditary syphilis, and in two cases of secondary syphilis, all being under mercurial treatment.

"If no change occurs after four or five days the reaction should accordingly not be termed negative and Noguchi advises that the observation of the patient be continued for at least three or four weeks.

"Neither in syphilitics nor in parasyphilitics did a marked constitutional effect follow the intradermic inoculation of the luetin. In most positive cases a slight rise in temperature took place, lasting

for one day. In three tertiary cases and in one hereditary case, however, general malaise, loss of appetite, and diarrhea were noted."

Results.—As regards the specificity and the value of the Noguchi reaction from a diagnostic standpoint there can be but little doubt, and it seems from the data which are thus far available that it is especially serviceable in the late stages of the disease, and in the recognition of congenital cases.

Noguchi expresses the belief that the allergic condition of the skin persists as long as the infecting agent still survives somewhere in the body, and that its disappearance, *ceteris paribus*, implies the cure of the patient. It is to be noted, however, that cases occur in which the disease persists in spite of treatment and in spite of the absence of the luetin reaction.

Kämmerer, who has recently repeated Noguchi's work, sums up his experiences as follows:

The intracutaneous reaction is devoid of danger and entails no special discomfort for the patient. Aside from one uncertain case it was specific for syphilis. A differentiation between the specific and non-specific traumatic reactions is possible in most though not in all cases. In cases of marked reaction the control site also often responds to the point of vesicle or even pustule formation. Of the cases examined which were known to be syphilitic more than half did not give the reaction, the highest percentage of positive findings occurring in late cases. In view of the occurrence of retarded reactions the patients should be observed for two weeks.

My own observations have been rather scanty. In the beginning, when 0.5 per cent. carbolic was used as a preservative, it was difficult to guard the preparation against contamination. With the use of the cresol the danger from this source is certainly less, but I must confess that a couple of very severe local reactions (very curiously most severe on the control side) have somewhat dampened my enthusiasm regarding the general applicability of the test. Further experience with it will be necessary before it can be recommended for routine use.

As regards the comparative value of the Wassermann and the luetin reaction it is still too early to make any definite statements.

For the present it will no doubt be advisable to control the one by the other.

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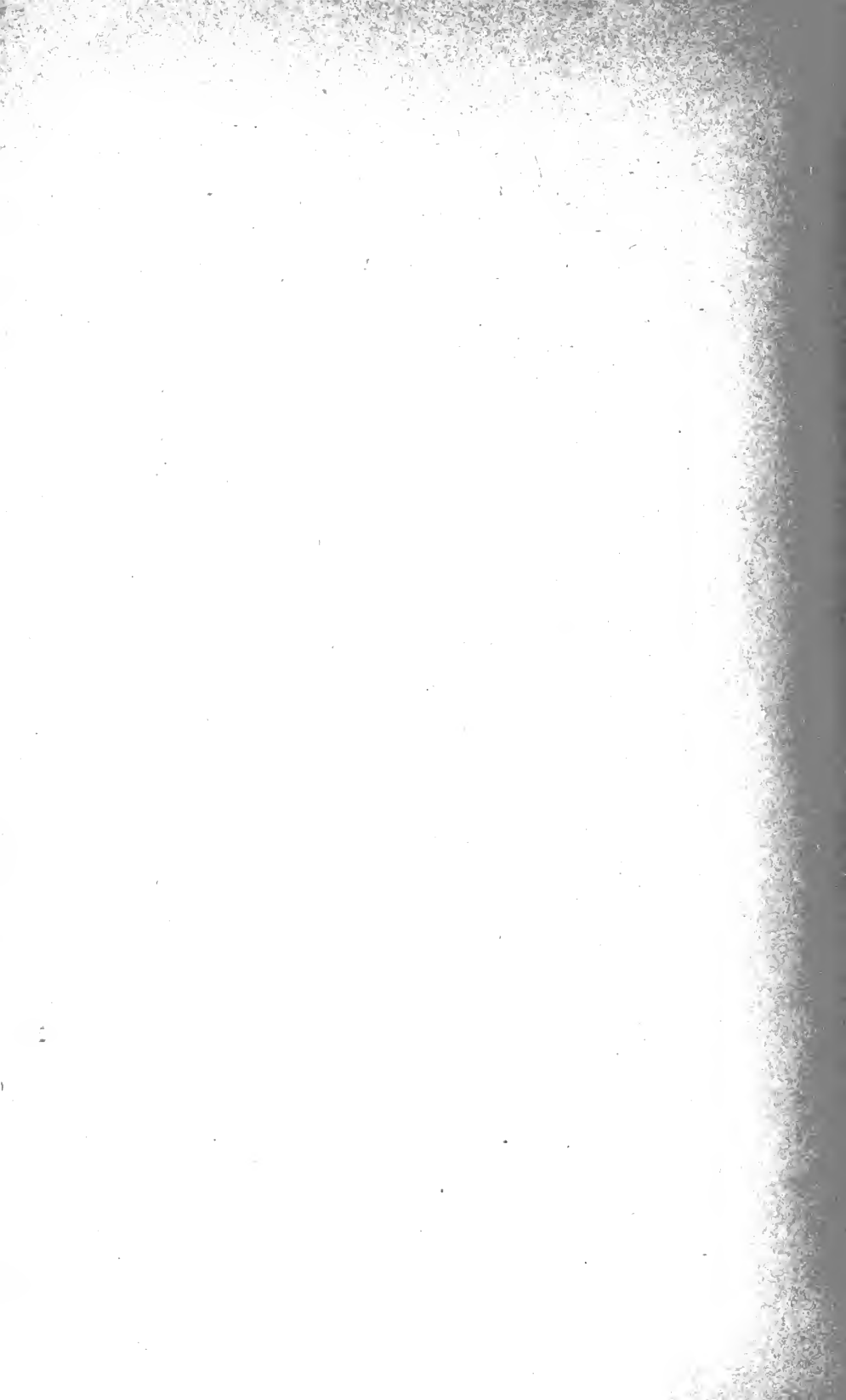
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